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The Effects of Pesticides on Human Health

Proceedings of a Workshop
May 9 - 11, 1988
Keystone, Colorado

Task Force of Environmental Cancer and Heart and Lung Disease

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16. ABSTRACT

This document presents the results of a workshop held from May 9 to 11, 1988, in Keystone, Colorado, focused on the potential chronic health effects of pesticides. Seventy expert participants collaborated within six working groups concentrating on pesticide exposure, neurotoxicity, carcinogenicity, immunotoxicity, developmental toxicity, and reproductive toxicity. This report combines the deliberations of each of these several working groups developed before, during and after the workshop. In addition, it includes a general introduction and overview of the subject and an executive summary that contains several conclusions and recommendations that represent the consensus opinion of the chairs of the working groups. The views expressed in this document are those of the participating scientists and should not be construed as representing any Agency position.

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PREFACE

The Task Force on Environmental Cancer and Heart and Lung Disease is an interagency group established by Congress in 1977. Congress mandated the Task Force to determine the relationship between environmental pollutants and human disease and to recommend research aimed at reducing the incidence of environmentally-related disease.

Pesticides are a class of chemicals that are introduced purposely into the environment for the benefit of controlling a variety of pest organisms. As a result of increasing national concern over the potential adverse health effects of pesticide residues in food and water and of pesticide exposures encountered occupationally, the Task Force sponsored a workshop on the effects of pesticides on human health.

The workshop, held on May 9-11, 1988, in Keystone, Colorado, focused on the potential chronic health effects of pesticides. Seventy expert participants collaborated within six working groups concentrating on pesticide exposure, neurotoxicity, carcinogenicity, immunotoxicity, developmental toxicity, and reproductive toxicity. This report combines the deliberations that each of these several working groups developed before, during, and after the Keystone workshop. In addition, it includes a general introduction and overview of the subject and an executive summary that contains several conclusions and recommendations that represent the consensus opinion of the chairs of the working groups. The views expressed in this document are those of the participating scientists and should not be construed as representing any Agency position.

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EXECUTIVE SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

Pesticides are developed specifically for their ability to interact and interfere with a variety of biological targets in the pests at which they are directed. Because of the fundamental similarities of organisms at the subcellular level, selectivity is often difficult to achieve and unanticipated pesticide actions leading to a number of adverse health effects may result.

Some occupational exposure to pesticides is unavoidable, even with strict adherence to good agricultural practices and proper application procedures. Members of the general population are exposed mainly through pesticide residues in food and water or through homeowner use of pesticides. Despite the availability of extremely sensitive analytical chemical capabilities, national data on human (especially general population) exposure to pesticides are lacking and are frequently rate-limiting in attempts to estimate potential adverse health impacts.

Although some data are available on the occurrence of acute pesticide poisonings and mortality, there is almost no information available on the incidence of adverse chronic health effects in humans. Very few epidemiological studies have been conducted in this area due, in part, to difficulties in identifying and quantifying exposures to different pesticides over time and the existence of many confounding factors. With the exception of the requirements in the State of California, there are no state, regional or federal laws that require the reporting of suspected pesticide-related illnesses.

The potential health risks from pesticide residues in food must be placed in proper perspective with the potential health risks associated with factors such as naturally-occurring plant substances, fungal mycotoxins and food-borne disease. Although we must continue to be vigilant with respect to pesticide residues in food, it is important that (with finite resources available) we do not overemphasize this at the expense of other, possibly far more important, risk factors associated with the U.S. food supply.

Workshop deliberations resulted in the following general conclusions:

1. Pesticides have the potential to cause a number of adverse chronic health effects in nontarget species, including humans.

2. Levels of human exposure to pesticides may be significant for certain occupational groups (formulators, applicators, farm workers, etc.) but are typically very low for members of the general population.

3. Studies with laboratory animals and *in vitro* model systems have established that pesticides have the ability to undergo a large number of mechanistically distinct interactions with a wide variety of different biological targets.

4. There is some concern that chronic adverse health effects in humans might result from excessive (high level, frequent, long-term) pesticide exposures that could occur in an occupational setting. However, although insufficient data exist, there is currently little evidence to suggest that such effects are likely to result from the traces of pesticide residues to which the general population is currently exposed.

5. There is no evidence that current levels of pesticide residues in food are causing any acute or chronic adverse health effects in the U.S. population.

The following recommendations were reached:

1. Immediate attention should be given to establishing a more comprehensive national data base to provide information on current levels and trends of human exposure to pesticides. Methods should be developed for measuring internal (blood and/or tissue) concentrations appropriate for risk assessment. Although a major goal should be to provide information on baseline exposure of the general population, attention should also be focused on potentially high risk populations (workers etc.) where exposures might be more easily linked with adverse health outcomes.

2. Given the heterogeneous nature of the potential chronic health effects of pesticides, there is an urgent need for additional research to determine the mechanisms by which chronic toxicity occurs, the most appropriate endpoints for identifying and measuring specific types of toxicity, and methods by which the results of animal studies can be extrapolated to humans. Potential adverse chronic health outcomes requiring attention include, but should not be restricted to, cancer, reproductive impairment (male and female), developmental effects (i.e., effects on the developing embryo), immunotoxicity (including hypersensitivity and asthma-type reactions), chronic neurological and neurobehavioral deficits.

3. Regulatory policy towards pesticides should strive to incorporate as much mechanistic information as possible in order to encourage basic research in this field.

4. There is a need for a more balanced level of concern and for a more equitable distribution of resources to identify and study all types of toxic effects. In recent years there has been a tendency to focus attention and resources on cancer at the exclusion of other endpoints of toxicity.

5. Although some information on proprietary products must remain confidential, compromise mechanisms should be sought to make the total existing data base (including industrial data) on chronic health effects of pesticides more readily available to the scientific community.

6. More epidemiological studies should be conducted on potential chronic adverse health effects in humans, especially in those populations of such as workers with known exposures to specific pesticides. Such studies should be carefully designed around clinical or other measurements based on toxicity endpoints or target organs established in animal studies.

7. Procedures should be developed whereby groups at potentially high risk to certain pesticides can be identified and protected. Such groups might include workers, pregnant women, infants, those with genetic predispositions or compromised immune systems or those receiving drug therapy.

8. More concerted efforts should be made at state, regional and national levels to establish a system that would require the reporting of all suspected acute and chronic pesticide-related health effects. This would ultimately result in the development of a comprehensive national data base.

9. Our knowledge base of the potential adverse health effects of naturally-occurring components of food is small relative to that of pesticides. More research should be conducted to identify naturally-occurring food constituents and to characterize their potential for causing adverse health effects in humans.

10. The extent of regulatory efforts directed towards identifying, measuring, and controlling pesticide residues in food must be considered in the context of the relative magnitude of the health risks of such residues versus the much greater risks associated with food-borne disease and naturally-occurring food constituents.

CHAPTER 1

INTRODUCTION AND OVERVIEW

C. F. Wilkinson (Chairman)[1]

INTRODUCTION

For most of civilization, man has waged a constant battle against the many thousands of organisms (insects, fungi, microbes, plants, etc.), termed pests, that compete directly for food and fiber, or pose a threat to health or general well-being.

In the course of this battle, many different weapons have been used with varying degrees of success. Early chemical insecticides included inorganic materials such as sulfur, arsenic, and mercury, and these were followed by several naturally occurring, plant-derived materials such as pyrethrum, nicotine, and rotenone. Various concoctions of lime sulfur and salts of copper and zinc were all that were available to fight fungal diseases, and weed control was effected predominantly by manual labor. These materials, along with a number of tars and oils (creosote, kerosene, etc.) and a few synthetic organics (dinitrophenols, thiocyanates) used for insect control, dominated the agrochemical arsenal until the time of World War II.

It was not until about 1940, when DDT burst onto the world scene, that the true potential of synthetic organic pesticides suddenly became apparent. DDT was quickly hailed as a panacea for insect control; and there is little doubt that during the 1940s and 1950s, worldwide use of DDT to control mosquitos and insect vectors of plant disease resulted in markedly reduced human suffering and enormous economic benefits to agriculture (Metcalf, 1973). During the following decade, it became apparent that many of these successes were not being accomplished without ecological damage.

Stimulated by the success of DDT, the chemical industry began an intensive search for other synthetic organic pesticides; and a steady stream of new insecticides, herbicides, and fungicides began to appear on the market. Production of inorganic materials, such as the arsenicals, declined as that of organics increased. By 1950, synthetic organic chemicals accounted for about 60% of the total pesticide market; this had increased to 90% by 1955 (Kohn, 1987); and the search for new chemicals continues to this day. Currently, the U.S. Environmental Protection Agency estimates that there are about 600 active pesticide ingredients marketed in some 45,000 to 50,000 formulations (Johnson, 1984).

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[1] In collaboration with Working Group Chairpersons.

CURRENT PESTICIDE PRODUCTION AND USE

According to the most recent available figures, U.S. production of pesticides and related products in 1987 amounted to about 1.04 billion pounds (100% active ingredients) (USITC, 1988). As indicated in Table 1-1, U.S. pesticide production has remained more or less level since the early 1980s, although there has been an annual decline of 5 to 8% in sales volume and value since about 1984. Despite these recent downward trends, sales levels are predicted to show a steady 5% increase through 1990. This is considerably lower than the 11.6% average annual growth that occurred between 1970 and 1985 and there seems little doubt that, while the agricultural chemical industry continues to do well, the overall demand for pesticide products is leveling off (Storck, 1987).

It is probable that the total pesticide production figures released by the U.S. International Trade Commission are underestimates, and it is difficult to break the figures down into amounts actually being used in the United States and those that are exported. Figures from 1980 indicate that about 800 million pounds were being exported and about the same amount was being used in the United States along with almost 100 million pounds of imports (Storck, 1980). For the last few years, it has been estimated that between 400 and 500 million kilos (0.9 to 1.1 billion pounds) of pesticide active ingredients are used each year in the United States (Pimentel and Levitan, 1986; CEQ,1986; USDA, 1982; Maddy, 1983; Young, 1987). Estimates for world production and use of pesticides are even more difficult to find but are generally indicated to be around 4 to 5 billion pounds (Maddy, 1983; Freed, 1987); world-wide sales of crop protection chemicals in 1989 are projected to be about $21 billion (Hunter, 1989).

Of the total pesticides currently used in the United States, about 60% are herbicides, 25 to 30% insecticides, and 10 to 15% fungicides (Pimentel and Levitan, 1986). This distribution is quite different from that existing in the early to mid-1960s when the amount of insecticides used was almost double that of herbicides (CEQ, 1986; Young, 1987). It is of interest that, according to Pimentel and Levitan (1986), about 80% of all herbicides currently used in the United States are applied to just three crops--corn (44%), soybeans

(25%), and cotton (9%); fruit and vegetable crops constitute the major uses of fungicides (about 60% and 24%, respectively).

The majority of the pesticides used in the United States (68 to 75%) are applied to agricultural lands (cropland and pasture), between 8 and 17% are used privately for control of household and home garden pests, and the remainder is utilized for industrial, commercial, and government purposes (Pimentel and Levitan, 1986; Maddy, 1983; Ware, 1983; Young, 1987).

TABLE 1-1. U.S. Pesticide Production and Sales[1]

Year	Production (billions lbs)	Sales	Sales Value (billions $)
1981	1.43		
1982	1.11		
1983	1.02		
1984	1.19	1.11	4.73
1985	1.24	1.02	4.44
1986	1.18	0.94	4.23
1987	1.04	0.91	4.17

[1] From annual reports on U.S. production and sales of synthetic organic chemicals compiled by U.S. International Trade Commission, Washington, D.C.

TYPES OF PESTICIDES

In addition to changing patterns in the amounts of pesticides being produced and used, rather dramatic changes have occurred in the types of chemicals that have been used since the start of the agrichemical revolution around 1940. In part, these changes have resulted from a changing philosophy concerning the role of chemicals in pest control and, in part, from concern over the potential adverse impacts of pesticides on the environment and human health.

The introduction of DDT and the chlorinated hydrocarbons had a profound impact on pest control practices and there was speculation that some important pests might even be eradicated. The chlorinated hydrocarbons were inexpensive, highly effective, broad-spectrum pesticides, and for a number of years they were used with relative abandon. Adverse effects became increasingly apparent, however. The stability and persistence of the organochlorine compounds in the environment, their accumulation in the tissues of living organisms, and their lack of selectivity were major factors in the development of pest resistance and in their deleterious impact on nontarget species (Carson, 1962). Their ability to undergo biomagnification through food chains caused serious problems for raptoral birds and carnivorous fish (Brown, 1978; McEwen and Stephenson, 1979), and there was concern over the potential effect on human health of organochlorine residues in human adipose tissues (HEW, 1969).

There is no question that the widespread use and misuse of the chlorinated hydrocarbon insecticides:

- raised public awareness of the potential adverse ecological effects of chemicals and triggered the U.S. environmental movement in the early 1960s;

- stimulated the enactment of Federal and state legislation to better protect human health and the environment from pesticides and other synthetic chemicals;

- led to the more rigorous testing of new pesticides and more careful review of existing materials; and

- challenged the pesticide industry to design and develop new pesticides with more acceptable environmental and toxicological properties.

DDT, which had been registered for use on some 334 crops and agricultural commodities in 1961, was banned in the United States in 1972, and the uses of most other chlorinated hydrocarbons were either banned or severely restricted during the next decade. While these actions undoubtedly had major ecological benefits, the resulting increase in use of the more acutely toxic organophosphorus compounds and carbamates increased the probability of human poisonings from accidental and/or occupational exposure (Davies et al., 1982).

The continued development of new and more effective pesticides that meet the more stringent modern standards of acceptability has become increasingly difficult, time-consuming and costly. The increasing costs for research and development and the decreasing chances of discovering a viable new product have severely strained the agrochemical industry. Through the 1960s, almost 20 new pesticide products per year were being introduced into the market at a cost of about $6 million per product. Since then, however, the rate of introduction of new materials has fallen precipitously and the costs have risen to over $40 million per product (Hollingworth, 1987). Recent estimates suggest that 25,000 to 30,000 compounds must be synthesized and evaluated in order to find a single commercial product (Storck, 1987).

The new pesticides now being introduced are sophisticated products that are the result of years of innovative basic research to enhance efficacy against undesirable pests. Thus, in contrast to DDT, which had to be applied at up to 5 pounds per acre, the new synthetic pyrethroid insecticides and the sulfonylurea herbicides require application rates of just a few grams per acre to be effective. The use of such small amounts of material clearly reduces the likelihood of residues in food and water and decreases the chance of accidental poisoning even though some, like the avermectin acaricides, exhibit high acute toxicity to mammals. The development of such highly active materials also is undoubtedly one reason for the apparent slowing (in terms of poundage) of United States pesticide production in recent years.

In addition, the new products are usually rapidly biodegradable and, as a result of more advanced application technology, are being applied more efficiently than was once the case. The days of automatic, prophylactic spraying have largely been replaced by carefully timed applications to meet the needs of well-designed, integrated pest management (IPM) programs.

RISKS AND BENEFITS OF PESTICIDE USE

Benefits

Pesticides have become an essential adjunct to modern agriculture in the United States and around the world. Indeed, many modern farming practices, such as the use of new cultivation techniques, large monocultures, and the new high-yielding crop varieties that are central to the success of the "green revolution" are made possible largely by the availability of pesticides. While the use of chemicals should not be considered the only method of pest control, pesticides remain the most widely used means of controlling the majority of agricultural pests.

Estimates of crop losses due to pests vary considerably. In the United States it has been estimated that, even with the use of pesticides, crop losses amount to about 30% or $20 million. Worldwide, pre- and postharvest food losses have been estimated to be as high as 45% (Pimentel et al. 1978; Ware, 1983). Results observed in treated and untreated plots of five crops (corn, soybeans, wheat, potatoes, and cotton) suggest that, without insecticide treatment, insect losses alone would average about 45%; insecticide treatment results in an average yield increase of about 36% (Ware, 1983).

While losses may be difficult to quantify, there is little doubt that, in the U.S., the use of pesticides results in increased availability, improved quality, and lower prices of a large number of fresh and processed agricultural commodities. Pesticides have also played, and continue to play, an important role in controlling insect vectors of a number of debilitating diseases of man and domestic animals. As a result of mosquito control programs, perhaps a billion people around the world are now free of the scourge of malaria. Similarly dramatic decreases have occurred in yellow fever, typhus, plague, Chagas disease, onchocerciasis, and sleeping sickness to name but some of the important arthropod-borne diseases that have caused untold human suffering and held back the economic development of large portions of the world.

Risks

Despite the undeniable benefits associated with pesticide use, a great deal of emphasis in recent years has been focused on the potential of pesticides for causing adverse effects on human health and/or the environment. The entire subject of pesticide use is one that has been embroiled in controversy for more than two decades. It continues to be a highly charged, politicized issue in which it is frequently difficult to separate fact from emotion. Pesticides have become a special focus of public concern because:

- they are synthetic chemicals that are released purposely into the environment;
- they are designed to be toxic to some forms of life and often have the potential to cause adverse effects in a number of nontarget species, including humans;
- humans are unavoidably exposed to pesticides either occupationally or involuntarily through pesticide residues occurring in food and water.

The initial concern, as clearly emphasized in the book *Silent Spring* (Carson, 1962; Marco et al., 1987), was for the potential adverse ecological impacts of pesticides. The primary focus was on DDT and other chlorinated hydrocarbon insecticides that, because of their environmental stability and persistence and their tendency to undergo biomagnification, were causing serious problems for a number of nontarget species. *Silent Spring* had a profound effect on the way in which pesticides were viewed by the general public, and its publication in 1962 signalled the start of the U.S. environmental movement.

During the last two decades, however, the main focus of public concern has shifted away from the overt environmental impacts of pesticides to the largely invisible problems related to the potential adverse effects of pesticides on human health (Ruckelshaus, 1985). Today, as a result of a combination of factors including public sensitivity towards health risks, an inadequate scientific data base, genuine scientific uncertainty and conflicting messages, a large segment of the U.S. public firmly believes that, indeed, pesticides do constitute a serious threat to human health.

Risk can be defined quite simply as the probability that some adverse effect will occur. In the case of a pesticide, risk is a function of the intrinsic capacity of the material to cause a given adverse effect (e.g., neurotoxicity, cancer, developmental, or immunotoxicological effects) and the level of exposure. Since pesticides are developed specifically for their biological activity or toxicity to some form of life and because at the subcellular level organisms have many similarities with one another, all pesticides are associated with a certain measure of risk. The degree of risk, however, will vary considerably, depending on the intensity and duration of exposure which, in turn, relate to the circumstances under which exposure occurs. The potential risks to a pesticide applicator or farm worker occupationally exposed to pesticides are likely to be greater than the risks to someone in the general population exposed only to traces of pesticides in food and/or water.

Pesticide Regulation: Balancing Benefits Against Risks

The manufacture, distribution, and use of agricultural chemicals in the United States are strictly regulated under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) enacted in 1947 and amended in 1972, 1975, 1978, 1980 and 1988. FIFRA, administered by the U.S. Environmental Protection Agency (EPA), requires that any pesticide registered in the U.S. must perform its intended function without causing "unreasonable adverse effects on the environment." The latter phrase is defined as meaning "any unreasonable risk to man or the environment taking into account the economic, social, and environmental costs and benefits of the use of the chemical." It is important to recognize that FIFRA is a risk-benefit statute. While use of the term "unreasonable risk" implies that some risks will be tolerated under FIFRA, it is clearly expected that the anticipated benefits will outweigh the potential risks when the pesticide is used according to commonly recognized, good agricultural practices.

The burden of providing the data to demonstrate that a given pesticide meets these registration requirements rests with the manufacturer. Current registration requirements include a comprehensive battery of tests on acute and chronic mammalian toxicity and on environmental transport, fate, and impact on nontarget species. Information on product composition, stability, and analytical methodology as well as data on residue levels likely to be found in any food crops on which it is to be used, are also required. The likelihood of leaching into groundwater is also evaluated. A separate registration must be approved by EPA for each type of application or for each crop on which the chemical is to be used. This information, along with the approved conditions of use and any special restrictions or hazard warnings, must be incorporated into the product's label.

In approving a registration, the EPA establishes a "tolerance" that is the maximum residue permitted in food for human consumption, or in feed for livestock, at the time that the crop is harvested, i.e., in the raw agricultural commodity (RAC). Tolerances represent the maximum residues that might occur at the maximum application rate allowed on the label. While food tolerances are set by EPA, they are monitored and enforced by the Food and Drug Administration (FDA) under the Federal Food, Drug and Cosmetic Act (FFDCA). The interaction between FIFRA and FFDCA leads to some interesting problems.

Section 408 of FFDCA requires that EPA should consider "the necessity for the production of an adequate, wholesome and economical food supply", in setting tolerances. Under this statute and the risk-benefit balancing requirements of FIFRA, it has not been unusual for EPA to register and set food tolerances for pesticides considered to be potential carcinogens. Section 409 of FFDCA, however, concerns tolerances of materials classified as food additives. This applies to pesticide residues only when the residue occurs following pesticide use during processing or when a residue present in a RAC is concentrated during processing. The problem with Section 409 is that it contains the Delaney Clause, which specifically prohibits the presence of residues of materials found "to induce cancer in man or animal." This creates a regulatory paradox that, while residues of "carcinogenic" pesticides are allowed in RACs under Section 408 of FFDCA, they are not allowed under Section 408. This legal inconsistency was the subject of a National Research Council Report (NRC, 1987) and legislative and regulatory efforts are currently being made to resolve the problem. Under EPA's new "negligible risk" approach to the regulation of some potentially carcinogenic pesticides, the cumulative upper-bound risks from all dietary sources will be fixed at a lifetime increased risk of one in a million (10^{-6}).

The 1972 amendments to FIFRA required EPA to review and reregister pesticide chemicals every 5 years. While a 5-year review has proved impractical, EPA is currently conducting a comprehensive review of "old" pesticide products, some of which were registered 30 or 40 years ago. Clearly, as indicated in an NRC report (NRC, 1984), the amount of toxicologic information available on many of these materials is outdated and inadequate to meet current registration requirements. EPA has been severely criticized for its rate of progress in reregistering this backlog of "old" pesticides; "adequate" data are available on only about 100 of approximately 600 pesticide active ingredients on the market. It should be pointed out, however, that reregistration is a slow and difficult process. EPA has given priority attention to those chemicals used on food crops in significant amounts (Moore, 1987) and by 1985 had completed review of pesticides accounting for 50% of the total poundage used in the United States; data currently in hand should raise this to cover about 80% of the pesticides used.

Residues of pesticides in drinking water are regulated under the Safe Drinking Water Act (SDWA) of 1974. In part this Act required EPA:

- to work with the National Academy of Sciences to develop a series of recommended maximum contaminant levels (RMCLs) -- subsequently called maximum contaminant level goals (MCLGs) in the 1986 Amendments to the SDWA -- that would serve as health-based goals for all types of drinking water contaminants;

- to develop maximum contaminant levels (MCLs) that are regulatory standards as close to the MCLGs as technically feasible; and

- to develop a series of health advisories (HAs) for emergency situations.

Until recently, when MCLs were proposed in National Primary Drinking Water Regulations for 30 synthetic organic chemicals (including 16 pesticides) (EPA, 1989), standards for very few pesticides had been established.

PESTICIDE EXPOSURE LEVELS

Knowledge of both the intensity and duration of exposure to pesticides is, of course, a critical prerequisite for assessing potential health risks. Unfortunately, despite the availability of modern analytical chemical capabilities, there is often as much (or more) uncertainty associated with assessing human exposure to pesticides as with evaluating potential toxicological effects.

Human exposure to pesticides may occur occupationally or may occur from any of several involuntary nonoccupational sources. While it is generally agreed that levels of occupational exposure are sometimes substantial and can be associated with a variety of acute and chronic health effects, solid experimental or epidemiologic data to support a relationship are seldom available. Much greater attention needs to be paid to assessing exposures and adverse health effects in population subgroups known to be exposed to pesticides.

Nonoccupational pesticide exposure among the general population appears to be low relative to occupational exposures. Probably the major source of pesticide exposure for the general population results from the use of pesticides in and around the home. About 90% of all U.S. households use pesticides, and there are ample opportunities for misuse and accidents. Other sources of exposure for the general population may be from air, food or water.

Contrary to widespread public belief, pesticide residues in fresh and processed food are typically low. The results of Federal (FDA) and state monitoring programs indicate that some 60-80% of all foods sampled (fruit, vegetables, grains, dairy products and meat) contain no detectable pesticide residues and almost all residues that are found are well within legal tolerance levels; only about 1% of the residues are considered illegal, mainly because they contain residues of pesticides not registered on that crop rather than because they contain residues in excess of the tolerance level. The results of FDA Total Diet Studies, which identify the levels of pesticide residues actually consumed by various age/sex population subgroups, show that total dietary intake is usually less than 1% of the acceptable daily intake for both domestic and imported products. It must be concluded that human dietary exposure to pesticide residues in the U.S. is consistently well within established safety standards.

The discovery, in the late 1970s, that ground water in some parts of the country was contaminated with pesticides created legitimate public concern, since about 85% of all water supplies in the U.S. are obtained from ground water aquifers that provide drinking water for about 50% of the U.S. population (95% in rural areas). In recent years, a good deal of regulatory and legislative action has been directed towards ground water protection at both the Federal and state levels (EPA, 1987). The EPA has initiated a national survey of pesticides in well water in an attempt to define more clearly the extent of the problem and has developed a "Pesticides in Ground Water Data Base", which pulls together the results of all ground water monitoring studies. The most recent interim report on this effort (EPA, 1988) indicates that some 46 pesticides have been detected in ground water in 26 states as a result of normal agricultural practices; 32 other detections in 12 states have been linked to point sources or misuse. In the majority of cases, the levels that have been found are below the EPA health advisories and, while still of concern, are not considered to constitute an immediate threat to human health.

In summary, while occupational exposures to pesticides should be considered a potentially serious problem, there are no data to suggest that pesticide exposures pose a significant health risk for the general population.

ADVERSE HEALTH EFFECTS OF PESTICIDES

Despite the large amounts of pesticide chemicals being used and the undoubted potential of many to cause adverse health effects in humans, there are still insufficient data to accurately determine the true impact of pesticides on human health. Even in the United States, where there has been so much concern and controversy about the health effects of pesticides, and where appropriate medical expertise and all necessary communication and data-gathering technology are readily available, there is still no centralized system for collecting information on acute pesticide poisonings.

Furthermore, very few reliable epidemiologic studies have been conducted with respect to either the acute or chronic adverse health effects of pesticides. Studies on the chronic effects of any particular pesticide are particularly difficult to conduct because agricultural populations are scattered and mobile and usually have a history of mixed exposure to many different pesticides and other confounding factors.

The primary focus of the present review is on the chronic health effects of pesticides. For the sake of completeness, however, it is also important to consider the extent and significance of acute pesticide poisonings.

Acute

Despite a good deal of speculation to the contrary, there is little evidence to indicate that, for most people, acute pesticide poisoning constitutes a serious problem in the United States. Possible exceptions to this are certain groups of agricultural workers whose occupations frequently bring them into direct contact with pesticides (Wasserstrom and Wiles, 1985). In recent years, however, it appears that improvements in general education, more effective regulations leading to improved pesticide labeling and packaging, and better applicator training and certification have led to a steady decline in cases of acute pesticide poisoning (Bonsall, 1985; Davies and Lee, 1987; Hayes and Vaughn, 1977).

Certainly, this appears to be the case with respect to pesticide mortality in the United States. Hayes and Vaughn (1977) carefully analyzed U.S. pesticide mortality figures for 1973 and 1974 and compared the results with those of previous studies (Hayes, 1976; Hayes and Pirkle, 1966). As indicated in Table 1-2, annual pesticide-related fatalities declined from 152 to 52 between 1956 and 1974. During the early part of this period, inorganic compounds, such as arsenic, cyanide and fluorides, were a primary factor in accidental deaths associated with pesticides (including suicide and murder); and it seems likely that the steady decline in the use of these materials during the last two decades has been responsible for the overall decline in pesticide-related deaths. The results of another study indicate an average of 35 pesticide-related deaths per year in the U.S. through the 1970s (Vital Statistics of the U.S., Vol II). A more detailed analysis of mortality data for 1973 and 1974 shows that approximately 70% were accidental, 20% due to suicide, and the remaining 10% the result of other factors (murder, misdiagnosis, etc.). The majority of accidental deaths involved either gross safety violations (e.g., improper use, packaging, or disposal) or incompetence (senility, drunkenness, illiteracy, etc.); sadly, some 30% of all fatalities in 1973 and 1974 were children under 10 years old. Occupational deaths in 1973 and 1974 were reported as 5 and 7, respectively (Hayes and Vaughn, 1977).

In 1976, EPA attempted to use 1971-73 hospital patient records to develop an accurate data-base on hospital-admitted pesticide poisoning cases and to use this to estimate nationwide incidence rates (EPA, 1976). Based on extrapolations from data obtained from a relatively small number of hospitals, it was estimated that, nationwide, there were fewer than 3,000 hospital-admitted pesticide poisonings per year. While mortality estimates of 49, 92, and 54, respectively, for 1971, 1972, and 1973 are similar to those reported by others, the EPA study provides no indication of the extent or severity of the nonfatal poisonings reported. The EPA's results were largely confirmed by another study (CPSC, 1985) suggesting that about 20,000 persons were taken to emergency rooms for actual or suspected pesticide poisonings each year and 10% of these were admitted for further treatment or observation. Based on about 48% of the nation's poison control centers, some 85,000 calls relating to pesticides were received in 1985; many of these reflected concern rather than actual illness and only about 24% received any medical attention. It is probable that the majority of real or suspected pesticide poisoning cases never go to the hospital and are treated at home or by a local physician.

Perhaps the most accurate statistics on acute pesticide poisonings are those from California where physicians are required by law to report all suspected pesticide-related cases and where many of these are subsequently evaluated by the California Department of Food and Agriculture (CDFA) (Maddy et al., 1979; Edmiston and Maddy, 1987). In recent years, the CDFA has received between 2,000 and 2,500 reports per year of suspected pesticide poisonings (Edmiston and Maddy, 1987; Maddy et al., 1979). Of these, slightly more than 50% result from confirmed occupational exposures to pesticides and 5 to 10% from confirmed nonoccupational exposure; some 40 to 45% of the reports cannot be related to pesticide exposure. A large number of nonoccupational poisonings are handled through state poison control centers and are not reported to CDFA. Thus, Maddy et al. (1979) estimated that there were about 14,000 incidents involving pesticide exposure each year. The vast majority of these, however, required little or no treatment.

TABLE 1-2. Pesticides Responsible for Accidental Deaths in Selected Years Between 1956 and 1974[1]

Pesticide	1956	1961	1969	1973	1974
Inorganic compounds	78	51	43	27	16
Organophosphorus compounds	17	23	18	9	18
Carbamates	0	0	1	1	1
Chlorinated hydrocarbons	4	3	3	3	1
Solvents, fumigants, propellants	1	3	1	5	4
Other compounds	52	31	21	16	12
Total	152	111	87	61	52

[1] Modified from data in Hayes and Vaughn (1977).

Most pesticide-related deaths in California seem to involve suicide. In 1977, for example, 12 or 13 of 18 deaths were attributed to suicide and 5 or 6 to accidents; there were no occupational deaths in 1977 (Maddy et al., 1979). Indeed, between 1975 and 1986, only 11 occupational deaths in California were related to pesticides; since some of these involved aerial applicators, it is uncertain whether pesticide exposure was directly to blame. To place these figures in proper perspective Edmiston and Maddy (1987) point out that, between 1976 and 1985, 130 California farm workers died in tractor accidents and more than 1,000 died in "on-the-job" auto and truck accidents.

The severity of the nonfatal poisonings is difficult to evaluate. Of the 1,065 confirmed occupational cases in California in 1986, 562 suffered systemic poisoning, 147 eye injury, and 342, skin problems. Thirty cases required hospitalization for a combined total of 99 days, and 268 cases claimed disability totaling 1,344 days (Edmiston and Maddy, 1987). In spite of increased Federal and state regulatory activity directed toward worker protection (required reentry times, protective clothing, etc.), it is clear that pesticide exposures of varying degrees of severity continue to occur. Unfortunately, it appears that in the United States and other developed countries (Bonsall, 1985), many cases of poisoning are due to gross misuse and negligence or to intentional exposure.

There remains an urgent need in the United States to develop a more effective mechanism for reporting the incidence and severity of both occupational and nonoccupational cases of pesticide poisoning. If systems similar to that existing in California could be developed by other states, these would readily enable the compilation of an accurate national data base.

It should be noted that, while the available data indicate that the extent of acute pesticide poisoning in other developed countries (U.K. and Europe, Australia, etc.) is relatively small, this is clearly not the case in many developing countries (Davies and Doon, 1987; Copplestone, 1985). Although worldwide estimates are very variable and highly uncertain, the World Health Organization has indicated that there may be as many as 500,000 pesticide-related illnesses per year with up to 20,000 deaths (Copplestone, 1977).

Chronic

In recent years, public concern over the potential adverse health effects of pesticides has focused increasingly on a series of chronic end-points including cancer, developmental, reproductive and immunological effects, and a variety of neurological and behavioral effects. To some extent, this concern has arisen from (and certainly has been exacerbated by) the fact that our basic understanding of the mechanisms by which chemicals can lead to many of these chronic effects is still in its infancy and that adequate test protocols by which they can be detected are either not available or have not yet been incorporated into pesticide registration requirements.

The primary purpose of this Workshop was to focus on these chronic adverse health effects and to address a number of basic questions relating to:

- the potential of pesticides to cause immunological, neurological, reproductive, carcinogenic and developmental effects;

- the likelihood that, and extent to which, these effects might occur in the human population under current exposure conditions;

- the availability of appropriate tests and/or monitoring capabilities to detect such chronic effects in animal or human populations; and

- major knowledge gaps and research needs.

These and related topics are discussed in detail in the respective sections of this report. The following constitutes a brief summary of the several reports.

Immunotoxic Effects. Based on the results of animal studies, several pesticides have the potential to modulate human immune system parameters and also to elicit contact dermatitis reactions in humans. The general absence of data to substantiate pesticide-induced immune suppression should not be interpreted as being indicative of the absence of potential risk to the immune system. The major factor is probably the exposure level required to elicit a response. Consequently, while effects might occur as a result of some occupational exposures, there is little or no evidence to suggest that pesticide exposure via the diet or drinking water is likely to compromise human health through interference with the immune system. The number of laboratory animal studies in this area is limited and essentially no studies have been conducted with humans. Indeed, to date, the immune system has been largely overlooked as a potential target for pesticide toxicity and a much more extensive evaluation is warranted.

There is definitive experimental and clinical data demonstrating that occupational exposures to some pesticides (e.g., captan and some carbamate and organophosphorus esters) induce contact hypersensitivity responses. However, the published animal test data (primarily guinea pig maximization data) tend to exaggerate the sensitization potential of most pesticides when compared against either reported clinical incidence of pesticide-related contact dermatitis or positive diagnostic patch test responses. On the other hand, the true incidence of pesticide-related contact dermatitis is likely to be somewhat greater than that reported due to inadequate tracking (diagnosis and/or epidemiology) of occupational adverse skin reactions. There is a need to improve the existing database on contact hypersensitivity.

The existence of population subgroups who can be considered "at risk" as a result of their relatively high occupational exposure to pesticides, provides an excellent opportunity for studying the potential immunotoxic effects of pesticides in humans; currently this is not being adequately utilized. Measurements of immune function should be conducted with such groups and compared against those of the general population even though the immune function parameters considered "normal" for the general population are not yet clearly defined. There is, as yet, little or no information available on how much immune dysfunction can be tolerated without incurring an adverse health effect or on the relationship between altered immune function and host resistance. There is a need for additional information on the mechanisms through which pesticides and other chemicals can cause immune dysfunction and hypersensitivity and on the ability of short-term tests (acute and subchronic exposures) to predict chronic immunological effects from long-term, low level exposures.

Neurotoxicity. As a result of the fundamental similarities between the vertebrate and invertebrate nervous systems, insecticides designed to attack the insect nervous system (organochlorines, pyrethroids, organophosphorus and carbamate esters) clearly are capable of producing acute and chronic neurotoxic effects in humans. Consequently, it is perhaps not surprising that both acute and chronic alterations in sensory, motor, autonomic, cognitive and behavioral functions have been observed in people exposed occupationally to relatively high levels of insecticides and some other pesticides. Occupational exposure to other pesticide classes has generated equivocal results and has raised questions concerning the ability of existing techniques to detect subtle changes in function. Thus, while human health can be affected adversely by exposure to herbicides, fungicides, fumigants and other pesticides, long-term neurological disabilities have neither been well documented in the literature nor studied systematically. While workers exposed occupationally to pesticides constitute the largest workforce in the world, they have received little or no attention with respect to the possible occurrence of adverse neurological effects. If alterations in neuronal function are not readily detected in this population, there is little chance that such changes would be observed in accidentally exposed bystanders or in the general population where pesticide exposure is limited to trace residues in food, air or water.

Neurotoxicity is not being adequately assessed by current methods of monitoring or testing (NHANES II and III, SENSOR, etc.) for adverse health effects in humans. Methods other than simple noninvasive measures of peripheral sensory and motor nerve conduction velocities are essential to detect and quantify subtle changes in neuronal function. Impaired vigilance, reduced concentration, memory deficit, linguistic disturbances, depression and irritability have been frequently reported clusters of symptoms associated with exposure to insecticides but have not been adequately assessed by current by current methodology. Reports of this type are often difficult to confirm are frequently anecdotal in nature and seldom followed up. The World Health Organization has recently proposed a 10-nation prospective, collaborative study of of worker exposure to organophosphorus insecticides that will employ a battery of tests to measure neuronal, neuromuscular and behavioral functions. Similar cooperative studies should be initiated between state and federal health agencies in the U.S. and Canada, particularly those states where intensive agriculture requires the heavy use of pesticides.

While we know a great deal about the acute neurotoxic effects of pesticides and other chemicals our current understanding of chronic effects leaves much to be desired. A more rigorous evaluation of chronic neurotoxic effects is required, based on sound scientific data from epidemiological studies as well as from experimental investigations using appropriate animal models. The methodology must utilize definitive endpoints of neurological function. Currently, a major gap in the assessment of neurotoxicity is in the application of existing cognitive and behavioral measurement techniques. Neurotoxicological studies should first be validated in appropriate animal models by using known, potent neurotoxic agents to characterize the problem(s) and to identify the most useful parameters for quantitative measurement. These studies should be followed by studies using less well described analogs or congeners to determine whether similar neurotoxic effects can be detected with these agents.

Reproductive Toxicity. Unlike other health effect endpoints that may be affected adversely by exposure to pesticides, reproduction is a couple-dependent biological process. All methods designed to assess reproductive risk in either animals or humans must consider this dependence on two individuals. For example, small decrements in reproductive function in one of a pair of experimental animals may not be manifest in changes in overall fertility. In humans, however, a slight decrement in a reproductive process in one member of a couple may be amplified and could cause infertility because of greater reproductive heterogeneity. In addition, reproductive processes in higher mammals have different sensitivities to perturbations than those in rodents.

A number of pesticides clearly have the potential to cause reproductive toxicity in animal species and several (e.g., ethylene dibromide, dibromochloropropane, kepone, carbaryl) have been shown to affect human (mainly male) reproduction following occupational exposures. Unfortunately, there is a paucity of published information that addresses, in a critical fashion, the effects of pesticides on reproduction and much of the data generated for the purpose of pesticide registration under FIFRA, for example, does not appear in the open literature.

There is a need to redesign present animal and human studies to define more explicitly the acute and chronic effects of pesticides on reproduction. A number of tests are already available and could readily be applied to pesticides. These include a more explicit evaluation of both the male and female gonad, and tests to determine time to pregnancy (indirect measure of failure to conceive and early pregnancy loss), reproductive behavior (libido and impotence) and menstrual cycle characteristics (provide noninvasive measure of hypothalamic, pituitary, ovarian and uterine function). A number of other tests could also prove useful with further development.

It would be advantageous to use several unrelated species of test animals and, because of unique anatomical and physiological characteristics, increased use of non-human primates would help to define mechanisms of action likely to be most relevant to humans.

Carcinogenesis. In recent years, there has been increasing public concern that long-term, low level exposure to pesticides in the form of residues in food and water might pose a potentially serious cancer risk to the general population. Concern has been heightened by recent reports (NRC, 1987; NRDC, 1989) indicating the existence of substantial risks associated with food residues of "carcinogenic" pesticides.

Insufficient data are available to estimate the pesticide-related cancer burden for the general population but it must be considerably smaller than that associated with other environmental factors such as smoking, radon and asbestos. The excesses that have been observed epidemiologically in humans have been in occupational groups where pesticide exposures are undoubtedly higher than those experienced by the general population. In these groups the excess for lung cancer (the most common cancer in the population) is small (less than 2-fold) and other cancers associated with pesticides are rare. Although many pesticides have not been evaluated in epidemiological studies, the available animal investigations indicate that most pesticides do not appear to be animal carcinogens.

Epidemiologic studies of occupational groups exposed mainly to some of the organochlorine insecticides have noted small, but consistent, excesses of lung cancer (less than 2-fold). Less common cancers such as those of the bladder, brain, lymphatic and hematopoietic system are less consistently elevated in these cohorts. Case-control studies have noted excesses of soft-tissue sarcoma, leukemia and lymphoma among farmers and foresters exposed to organochlorine insecticides such as DDT, chlordane and lindane. Positive findings from DDT and chlordane are available from animal bioassays but both are negative in the Ames test indicating that these materials are not genotoxic. Several organochlorine insecticides have been shown to inhibit intercellular communication and to act as tumor promoters.

Several case-control studies and cohort studies of herbicide manufacturers and applicators have noted associations between phenoxyacetic acid herbicides and soft tissue sarcomas and non-Hodgkins lymphoma. The phenoxyacetic acids have not been reported to be animal carcinogens in chronic bioassays although they can serve as peroxisome proliferators in animals, an action that might play some role in carcinogenesis.

Most pesticides that are carcinogenic in laboratory animals affect a limited spectrum of target sites; a few pesticides such as captafol, dibromochloropropane, tetrachlorvinphos, trefluralin and ethylene oxide appear to induce cancers at multiple sites in some species. However, the target tissues or organs associated with pesticide-induced tumors in animals are not consistent with those observed epidemiologically in humans. For example, some epidemiologic studies have associated lung, bladder, soft-tissue sarcomas and lymphatic and hematopoietic cancers with exposure to the organochlorine insecticides while, in mice, these pesticides are associated almost exclusively with liver tumors.

Pesticides have received considerable attention from both experimentalists and epidemiologists for a number of years. Both approaches are necessary and each makes an important contribution to our understanding of this issue. The apparent inconsistencies between experimental and epidemiologic results point to a need for modifying our current research strategy. First, many more pesticides have been evaluated for carcinogenicity in animal bioassays than have been studied epidemiologically. Although numerous occupational groups with routine exposure to pesticides can readily be identified, the majority of the individuals involved have concomitant exposures to many different materials. Clearly, this complicates the evaluation of any one particular pesticide. Different pesticides, however, are targeted at specific pests and it should be possible to identify situations in which groups of workers are exposed primarily to one pesticide. Experimental studies typically focus on a single pesticide but do not mimic the human exposure situation. While it would be advantageous to utilize an experimental approach that more closely reflects the conditions of human exposure (i.e., intermittent multiple exposures to several pesticides) this would prove very difficult to implement.

The classical paradigm of carcinogenicity for both experimentalists and epidemiologists alike has been that chemical carcinogens are electrophiles and mutagens and act via a direct genotoxic mechanism. According to this paradigm carcinogenic pesticides would all be genotoxic and act in the early stages (initiation) of the carcinogenic process. There is increasing evidence, however, that chemicals can cause cancer by any of a number of nongenotoxic mechanisms such as promotion, peroxisome proliferation, hormone imbalances and cytotoxicity leading to compensatory cell division. While a number of pesticides are classified as animal carcinogens, it is likely that many of these are acting via nongenotoxic mechanisms.

As our understanding of the mechanisms of carcinogen action has improved, there has been corresponding modifications in study design and interpretation. This is a continuing process and the time may be ripe for additional modifications that can accommodate the rapid advances occurring in the area of mechanisms of action. There is a need, for example, in both experimental and epidemiologic investigations to develop the capability of evaluating effects at different stages of the carcinogenic process. In epidemiologic studies, risks should be evaluated with respect to recent exposure and not just by the prevailing view that only the earliest exposures are relevant. In experimental studies there is a need to develop the capability of assessing promotional activity in addition to evaluating initiation potential. Failure to make such modifications may result in false negatives or "animal positives" of uncertain relevance to humans. Either circumstance would place severe limitations on our ability to make sound scientific and societal decisions.

Developmental Effects. Developmental toxicology is the field of science exclusively devoted to the study of adverse effects of chemicals on the conceptus; it covers the period from conception to the completion of morphologic structure and functional capability that probably does not occur until puberty. Development is an exceedingly complex process and its sensitivity to chemicals as well as the manifestations of toxic exposure vary with the developmental stage at which exposure occurs. The conceptus is most vulnerable to the initiation of major birth defects between three weeks and two months, the critical period of organogenesis. Exposures to toxic chemicals during the first two weeks following conception typically leads to fetal death, while exposure later in development is more likely to cause growth retardation and functional deficits of various organs.

The theoretical potential of pesticides to cause adverse effects on development have been demonstrated quite clearly in animal experiments where a full array of effects have been observed at various stages of development. Of some 200 pesticides tested so far, nearly half are reported to have some capacity to induce birth defects in experimental animals. This is not unusual since virtually all bioavailable chemicals will interfere with some aspect of development if administered at a sufficiently high dose level under appropriate conditions. Thus, hazard identification hinges mainly on the dose level at which an effect is observed and, particularly, on whether the adverse effect on the conceptus occurs at exposure levels below those causing severe maternal toxicity. Developmental toxicity testing protocols almost always use high doses, whereas in general, humans are exposed to low doses. As in other areas of toxicology, the extrapolation of effects observed at high doses to predict those likely to occur at low doses and the extrapolation from animal data to humans, are two serious problems that currently limit efforts to assess the potential developmental risks pesticides and other chemicals.

The failure to find significant human developmental toxicity is neither surprizing nor reassuring. Finding a cohort of individuals exposed to a single pesticide is exceptionally difficult. Furthermore, since human malformations occur in roughly 5% of live births, demonstrating an increase in the overall rate of malformation or the incidence of a specific type of malformation from a documented exposure requires a much larger population than if the background rate were zero.

ASSESSING CHRONIC HUMAN HEALTH RISKS

It is somewhat disconcerting to realize that, while there are reasonably reliable data on pesticide-related deaths and to a lesser extent acute non-fatal poisonings, information on chronic illness or morbidity resulting from human exposure to pesticides is almost nonexistent.

Information on the potential adverse effects of chemicals on human health is obtained by either extrapolation from experimental studies on laboratory animals or from direct clinical and epidemiologic observations in human populations; both approaches are important. Experimental studies are usually prospective in nature and provide valuable information on the mechanisms of chemical toxicity and sensitive clinical or other end-points that can be used to indicate the onset (or degree of severity) of specific types of toxicity. Epidemiologic studies are typically retrospective and at the present time are often limited to crude end-points of toxicity such as death, cancer and severe neurological disease. Their success and sensitivity would be enhanced immeasurably by the availability of more sensitive indices of early toxicity that could be correlated with exposures to specific chemicals.

As discussed in the previous section, many pesticides have the potential under certain conditions, to cause a variety of adverse health effects. Scientific understanding of the mechanisms through which these occur is rapidly increasing. Particularly in the case of chronic toxic effects there is an urgent need to continue basic mechanistic studies and to develop new and more sensitive early end-points of different types of toxicity. This is by no means a simple undertaking and even if such tests can be developed it will be necessary to define the "normal" range of such parameters (Kimbrough, 1987).

The general absence of specific indices of chronic toxicity, the extremely low and variable exposure to pesticides, and the presence of a multitude of confounding factors combine to make meaningful epidemiologic studies in the general population difficult. Clearly, there is a need to focus additional studies on carefully selected "high risk" population subgroups who are known to have relatively high levels of pesticide exposure (plant employees, formulators, applicators, agricultural workers, etc.).

It seems unlikely that broad national surveys such as the National Health and Nutrition Examination Survey III (NHANES III) (HHS, 1988) or that of the Agency for Toxic Substances and Disease Registry (ATSDR) (ATSDR, 1988) will be adequate to identify pesticide-related health effects. Thus, while the former includes a wide variety of diseases or medical conditions and emphasizes several risk factors and population subgroups (children, senior citizens, occupational groups, etc.) (HHS, 1988) it is not likely to be sufficiently sensitive to pick up chronic pesticide-related effects, and the latter is focused primarily on potential health problems associated with exposures to hazardous substances in waste sites and chemical spills. The National Human Adipose Tissue Survey (NHATS), operated by the EPA, is designed to analyze a nationwide sample of human adipose tissue for the presence of a variety of toxic substances, including some pesticides. This survey, however, is restricted largely to highly lipophilic, persistent materials such as the chlorinated hydrocarbons and is of little value in measuring body burdens of more hydrophilic pesticides or those that are rapidly metabolized and excreted. Furthermore, since all samples of adipose tissue are obtained from surgical patients or cadavers, there is some question as to whether they are truly representative of the general population.

PESTICIDES VERSUS OTHER HEALTH RISKS

General

Many people associate pesticides with a level of risk considerably greater than that suggested by existing data (Upton, 1982). Although even a single fatality is too many, pesticides appear to constitute a very minor cause of accidental death relative to other familiar causes, even when compared with other poisonings. Thus, in 1983, pesticides were reportedly responsible for only 22 accidental deaths relative to some 1,189 from carbon monoxide and other gases and almost 3,000 from medicinal substances such as aspirin (National Safety Council, 1985). Occupationally-related deaths involving pesticides also appear to be small compared with deaths associated with many other occupations although, as previously discussed, data on the extent of chronic illness from pesticide exposures are currently incomplete.

Food

Recently, there has been widespread national concern over the potential health risks to the general public of traces of pesticides or their metabolites found in a variety of raw and processed foods. This concern, focusing particularly on cancer and reproductive toxicity, has resulted in part from intense media coverage of information contained in a National Research Council report (NRC, 1987) and in a more recent report published by the Natural Resources Defense Council (NRDC, 1989). Each of these reports associated relatively high cancer risk estimates with consuming fresh and processed fruit and produce containing pesticide residues. Unfortunately, the risk estimates of the NRC were based on unrealistically high theoretical levels of pesticide intake, and those of the NRDC were seriously flawed as a result of a number of conceptual and procedural errors (Wilkinson et al., 1989). As discussed earlier, the levels of pesticide residues occurring in food at the time it is actually consumed are very much lower than commonly believed. It is somewhat ironic that the major focus of public concern is on a group of chemicals (pesticides) that play such a dominant role in determining the quality, quantity, variety, and cost of a large portion of our food supply.

The current situation with respect to the food supply appears to be well summarized in a recent report from the Institute of Food Technologists (IFT, 1989). This report, endorsed by some 14 scientific societies concluded, in part, that:

- "The perception that the food supply is unsafe is not supported by scientific data; the American food supply is among the safest in the world.
- The scientific community needs to communicate the concept of risk more effectively to the public.
- Current allocation of resources devoted to food safety is not commensurate with actual risks but more with risks as perceived by the public."

The report went on to point out that current scientific evidence indicates that any health risks associated with pesticide residues in foods are orders of magnitude less than those of food-borne disease and insignificant relative to those associated with naturally-occurring chemicals in foods (IFT, 1989). Indeed, it has been generally accepted for many years (Wodicka, 1985) that, in order of priority, the major risks in the food supply are associated with:

- Food-borne disease

- Malnutrition
- Environmental contaminants (natural and man-made but not pesticides)
- Naturally-occurring toxicants
- Pesticides, food additives, etc.

The following brief discussion of some of these factors is intended to place the health risks from pesticide residues in food into better perpective.

Food-borne Disease. In contrast to the situation with pesticides, there is considerable evidence that food-borne disease, mainly of microbiologic origin, constitutes by far the major health hazard associated with the food supply. It is estimated that up to about 6 million cases of food- and water-borne disease causing some 9,000 deaths may occur in the U.S. each year (Archer and Kvenberg, 1985; Hauschild and Bryan, 1980; Todd, 1985; Bennett et al., 1987). The economic costs of these diseases have been estimated at between $1 and $10 billion per year (Todd, 1985).

Salmonellosis and campylobacteriosis are undoubtedly the most serious foodborne diseases in the U.S., each causing as many as 2 million cases and 2,000 deaths per year. Despite this, the fact that about a quarter of the fresh poultry sold in this country carry Salmonella seems to cause only a ripple of public concern and is deemed to warrant minimal regulatory attention. Other important causes of food-borne enteric disease include *Shigella*, *Straphylococcus aureus, Clostridium perfringens, Yesinia enterocolitica, Bacillus cereus, Escherichia coli*, and *Vibrio parahaemolyticus*. While not affecting large numbers of individuals, botulism (*Clostridium botulinum*) continues to kill several people each year, and there have been several recent outbreaks of listeriosis (*Listeria monocytogenes*) from contaminated dairy products. The extreme seriousness of listeriosis is shown by the fact that there were 19 deaths out of 49 people infected during an outbreak in Massachusetts in 1983, and 80 deaths from several hundred people who consumed contaminated cheese in California in 1985.

Environmental Contaminants. This is an ill-defined group of materials of natural or technologic origin that includes radionuclides, industrial or municipal wastes, combustion products, and heavy metals. The extent of the problem of food contamination of this type is difficult to assess. It may involve the contamination of large ocean fish with mercury, selenium toxicity from grain grown on selenium-rich soils, or contamination of food from accidental chemical spills or misuse of household chemicals.

Naturally-occurring Toxicants. Contrary to the popular belief that everything natural is good and wholesome, the plant and animal kingdoms are replete with examples of materials containing a wide variety of naturally-occurring toxins.

Improper storage of some commodities infected with several types of mold before harvest can lead to the production of mycotoxins, some of which are extremely toxic systemically or carcinogenic. Perhaps the best known mycotoxin in the U.S. is aflatoxin, which is a common contaminant of wheat, corn, nuts, and a variety of other stored carbohydrates. The aflatoxin content of U.S. peanut butter averages about 2 ppb and is allowed by the FDA up to a level of 20 ppb. Aflatoxin is highly toxic and is a potent mutagen and carcinogen. It is likely that many more of the over 300 mycotoxins that have been characterized are also potent toxicants.

Some naturally occurring toxicants, mainly those leading to rather "spectacular" acute toxic effects, have long been recognized and are usually successfully avoided. Even so, there are still many people each year who suffer serious health effects, even death, as a result of eating poisonous mushrooms (e.g., *Amanita* sp.) and, in some parts of the world, ciguatera poisoning from consuming fish contaminated by a dinoflagellate toxin is a serious problem (Ragelis, 1984). Other potent natural toxins encountered from time to time are saxitoxin, associated with the so-called "red tide" and causing paralytic shellfish poisoning, and tetrodotoxin from the puffer fish. Serious acute poisonings may also occur as a result of consuming amygdalin-containing peach or apricot kernels that release cyanide on hydrolysis by the normal digestive enzymes.

In recent years, however, the major focus of attention has been on the apparent plethora of mutagens and other toxicants that are being detected with increasing frequency in a wide variety of fruit and vegetables considered normal dietary components (Ames, 1983). These secondary plant substances, thought to have evolved as "nature's pesticides," frequently make up 5 to 10% of the dry weight of the plant and are now considered by some to be the main source of toxic chemicals ingested by humans (Ames, 1983; Ames et al., 1987; Ames and Gold, 1989; Beier, 1989). Indeed, it is estimated that, of the "pesticides" we eat, some 99.99% are of natural origin (Ames and Gold, 1989). While, as yet, very few natural products have undergone vigorous toxicity testing, there is every indication that they will exhibit a range of types and potencies of toxicity similar to those of synthetic chemicals; to date, about half (20/42) of the plant toxins tested have been shown to be carcinogenic in animal bioassays (Ames and Gold, 1989). Clearly, in considering the potential risks associated with the food supply, there is a need to pay more attention to naturally-occurring toxicants and allocate more resources to identifying and evaluating the potential effects of such materials.

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CHAPTER 2

EXPOSURE TO PESTICIDES

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> *What is there that is not poison? All things are poison and nothing (is) without poison. Solely the dose determines that a thing is not poison.*
>
> -- Paracelsus, 1492-1541
> (Deichmann et al., 1986)

INTRODUCTION

Exposure assessment integrates information about released quantities of a substance, modes of application, behavior in the environment, direct and indirect channels to humans, duration of contact with portals of entry into the human body, metabolism, estimation of dose to target tissue, and relevant characteristics (age, sex, lifestage, race, etc.) of the receptor population. This information is used by risk assessors to judge the likelihood of injury and to assess cause-effect relationships between dose and disease incidence.

Exposure evaluation is a critical component of the risk assessment process. A specific pesticide may be extremely toxic; however, an exposure evaluation might determine that a person would be overexposed only in the case of equipment failure. A product could be found to be of low toxicity, but its common use might involve long hours of exposure to many workers and thus greater hazard. The basic toxicology data for a product might indicate a moderate toxicity; however, the proposed use of the product could lead to conversion of the active ingredient to a more toxic compound on leaf surfaces, constituting a hazard to field workers. Consequently, it is important to determine the magnitude of the exposure to the worker and to assess whether it could potentially result in unacceptable health effects.

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The exposure evaluation might include consideration of:

1. The use pattern (geographic, climate, season, equipment type, etc.) of the proposed product;

2. The likelihood of significant human exposure;

3. The amount of pesticide contacting body surface areas (skin, lungs, gastrointestinal tract) and the amount actually absorbed;

4. The adequacy of regulations or label instructions to inform users of the possible hazards of use and how to avoid excess exposure;

5. The adequacy of information provided to recognize illness due to exposure if it occurs;

6. The adequacy of first aid information; and

7. The availability of data to support medical management.

After all relevant data are fully evaluated, an assessment is made regarding the adequacy of measures taken to protect workers from possible hazards of use. The use instructions on the label might be accepted and the product registered without further concern. Conversely, one or more of the following conditions might be required before the product is eligible for registration: (1) the registrant might be required to make a label change to provide a warning or more specific use instructions; (2) the product might be regulated or restricted by the imposition of specific exposure reduction measures, such as (a) a closed system transfer of liquid pesticides; (b) a change in the product's formulation to reduce excess exposure (e.g., reduce dustiness); (c) water-soluble packaging to obviate the need for human handling; (d) minimum field reentry intervals; (e) medical supervision; or (f) detailed safety training.

ROUTES OF EXPOSURE TO PESTICIDES AND THEIR DISPOSITION IN THE BODY

For the majority of persons, exposure to and absorption of pesticides occur primarily through the skin. Ingestion of synthetic pesticide residues in food and water is typically low, as is inhalation of pesticides other than those that are volatile (e.g., DDVP and fumigants). Low-level inhalation exposure may be continuous in homes and office environments where pesticides are used. The relative importance of exposure routes for pesticide workers is similar.

During August, 1986, the 24-hour mean concentration of chlorpyrifos in air of the average Jacksonville, Florida, residence that had been sprayed with the chemical was 0.3 $\mu g/m^3$ (as determined by personal air sampling), providing an average daily inhalation exposure of 13 μg/day. Eight months later, personal exposures to chlorpyrifos were found to be reduced by only one-third (to 0.2 $\mu g/m^3$). In this case, the estimated dermal dosage was 40 times greater than the average daily respiratory exposure; however, such applications will not be expected to occur frequently in most households.

The efficiency of dermal penetration depends on the chemical type, concentration, and physical form of the pesticide product. Although some pesticides (especially organophosphates) have been shown to penetrate the skin readily (Feldmann and Maibach, 1974), EPA assumes in exposure assessments 10% dermal absorption for liquid and spray formulations and 1% absorption for granular forms and dusts (Jensen, 1984). More recent studies in test animals have shown that dermal absorption rates can range from less than 1% to over 90%. It is generally assumed that 100% of the pesticide reaching the lungs or the gastrointestinal tract is absorbed, although this is not always the case.

Davis (1984) estimated that during homeowner applications of spray pesticide formulations to the lawn, it is not uncommon for the hands to receive more than 90% of the total dermal exposure. Similar findings were reported by Reinert and Severn (1985) for agricultural workers in mixer-loader operations. Kurtz and Bode (1985), however, found greater exposures to the lower body extremities during home garden treatments. Using carbaryl as a model, they estimated an average total exposure of 8.5 mg, primarily to the feet and lower legs. Inhalation exposure was judged to be negligible. Wolfe (1976) also considered dermal exposure during application to greatly outweigh inhalation exposure. The use of rubber gloves and long clothing while mixing and applying pesticides might eliminate 95% of the potential dermal exposure (Kurtz and Bode, 1985).

Dermal exposure to household pesticides is currently being estimated by EPA in its Non-Occupational Pesticides Exposure Study (Lewis and Bond, 1987). In a worst-case situation, a study participant applied granular chlorpyrifos formulation by hand, leaving a residue of 50 mg of the insecticide on gloves provided by EPA. Personal air monitoring during the short (approximately 5-minute) application indicated that he was exposed to average air levels of 13.4 $\mu g/m^3$. The participant would have been exposed to a 0.4 μg inhalation dose (assuming a light-work respiratory rate of 30 liters/minute) and 500 μg dermal dose (assuming 1% absorption).

The greatest dermal exposure around the household may be to infants and toddlers. Berteau et al. (1989) calculated "worst case" exposure doses for a six- to nine-month-old child crawling around on floors or carpets containing 12.9 mg/m^2 of dislodgeable pesticide residue left from indoor insecticide treatments (e.g., for fleas). Assuming 20% to 100% dermal adsorption, they estimated exposure doses of 2.2 mg propoxur per kg of body weight, 50 mg/kg dichlorvos, and 2.7 mg/kg chlorpyrifos, of which about 90% would occur via skin penetration, 9% to 10% orally (from licking hands), and less than 1% by inhalation. Even if dermal adsorption estimates were reduced to 10%, the dose through the skin would be equal to or greater than the oral dose.

The Biologic and Pharmacokinetic Basis of Pesticide Disposition in the Body

Information on tissue-specific biologic disposition of pesticides in humans could offer the potential for more accurate information about the actual dose and biologic impact of pesticide at target sites of toxicity. This information would provide a better estimate of actual exposure for the purpose of risk assessment than the gross exposure dose which is merely the amount of chemical applied to, inhaled or ingested by the subject.

Biologic disposition of pesticides in humans concerns their entry into the body, and subsequent distribution, biotransformation and elimination (Gibaldi and Perrier, 1982). The time course of these processes, termed interchangeably pharmacokinetics or toxicokinetics, can be a basis for linking the magnitude of external exposure of the pesticide to the internal exposure of specific tissues to the pesticide or its derivatives. This may provide a basis to link external exposure to observed pharmacologic effects via a pharmacodynamic model. The latter relates tissue concentrations of a pesticide or metabolic derivative to the intensity of pharmacologic action(s) (Holford and Sheiner, 1981).

Pesticides may enter the body by inhalation, dermal absorption, or oral ingestion. Two key parameters characterizing the pesticide entry profile are the rate and extent of bioavailability. Extent of bioavailability refers to the mass of pesticide actually reaching the body's general circulation from a given site of entry. Referenced to the amount of pesticide presented to the site of entry, the extent of bioavailability is usually expressed as a percentage. The rate of bioavailability may be expressed as a constant when exposure is via breathing of pesticide-laden air. A first-order rate constant or half-life of absorption may be more appropriate when pesticide entry is through gut absorption; whereas, skin diffusion or permeability coefficients are sometimes used to characterize dermal entry characteristics.

A sizeable knowledge base exists regarding chemical permeation of skin and can be expressed by several principles: (1) most chemicals pass through skin by the first-order process of passive diffusion; (2) for most chemicals the stratum corneum is considered to be the rate-limiting barrier in skin; (3) the rate and extent of absorption across this barrier is inversely related to molecular weight and water solubility, but directly related to fat solubility; (4) hair follicles and sweat glands occupy only a small fraction of skin surface area and, therefore, likely play only a minor role in excretion and absorption; and (5) the metabolic capacity of skin may affect bioavailability (Bronaugh and Maibach, 1985; Scheuplein and Bronaugh, 1983).

The route of pesticide entry is a major factor affecting the amount of the exposure dose that actually reaches the target tissue as the biologically available dose. For example, pharmacokinetic studies of picloram in human male volunteers showed that a 5 mg/kg dose of this pesticide was rapidly and almost completely bioavailable upon oral ingestion, 93.8% of the ingested dose being excreted in the urine within 72 hours. In contrast, according to urine data, only 0.18% of a topically applied dose entered the body transdermally, and entry at this site was much slower than the oral route (Nolan et al., 1984).

Even for a single route of exposure, the entry profile may be affected by various conditions associated with the exposure. For example, absorption of 8-methoxypsoralen (8-MOP)--a chemical which has been used to treat psoriasis and also is a phytoalexin in celery--is slower when taken with food than when taken on an empty stomach; however, the areas under the serum-concentration-time curves for both administration schedules are the same, indicating that overall extent of bioavailability of 8-MOP is not affected by food intake (Herfst and De Wolff, 1982).

For percutaneous absorption, studies have shown that bioavailability of many pesticides increases with concentration, surface area of application, duration of contact, and degree of occlusion by outer garments. Even the anatomical site of application can dramatically influence absorption. For example, 36% of a standard amount of parathion was absorbed from the human forehead, but only 8.6% from the forearm (Wester and Maibach, 1985).

Pesticide distribution within the body can also be viewed in terms of rate and extent. When physiologically based pharmacokinetic models are employed (Gerlowski and Jain, 1983), the time course of pesticide distribution into organs or lumped tissues is expressed in terms of physiologic blood flow rates and partitioning into anatomically correct volumes at sites of distribution. For example, D'Souza and coworkers (1987) modeled the distribution of ethylene dichloride into lungs, liver, richly and poorly perfused tissues, and fat. Partition coefficients were determined experimentally; tissue volumes and blood flow rates were determined from allometric equations. When compartmental pharmacokinetic models are used (Gibaldi and Perrier, 1982), distribution rates are typically expressed as first-order rate constants or distribution half-lives, and extents of distribution are given as single or lumped compartment volumes referenced to the observed plasma concentration.

Pesticides can be metabolized by hydrolysis, oxidation, or conjugation processes that generally transform them into less toxic water-soluble forms, excretable in urine or bile. Alternatively, pesticides can be biotransformed to derivatives responsible for toxic effects. Although the liver is a primary site of pesticide biotransformation, other tissues, including the kidney, skin, plasma, and gut, contain metabolic enzymes responsible for pesticide biotransformation (Gram et al., 1986; Noonan and Wester, 1985). Pekas (1980), for example, has reviewed the possible role of gut metabolism on pesticide carbamates. Irreversible biotransformation is not a necessary prerequisite for involvement of a pesticide in a toxic metabolic process. For instance, although the pesticide paraquat does not appear to undergo any permanent structural biotransformation, an unusual chemical sequence in the lung plays an important role in its toxicity. This pesticide accumulates in the lung through a diamine transport process in the alveolar epithelial cells and Clara cells of the airways. In the lung, paraquat cycles through oxidized and reduced forms, producing free radicals and depleting NADPH--processes that may lead to its toxicity (Smith, 1987).

The extent of pesticide metabolism is usually expressed as the percentage of the bioavailable pesticide mass that can be accounted for as various biotransformation products excreted following a known pesticide exposure. To illustrate, Knaak and coworkers used high pressure liquid chromatography with fluorescence detection to analyze the urine of male volunteers who ingested 2 mg/kg carbaryl. They found that 26% to 28% of carbaryl equivalents could be accounted for by three metabolites: 4-(methylcarbamoyloxy)-1-naphthyl glucuronide; 1-naphthyl glucuronide, and sulfate. (Knaak et al., 1968).

Metabolism is inherent to the concept of bioavailability, for it may influence the amount of substance reaching the general circulation. Pesticides may induce or inhibit the enzymes associated with metabolic processes. Both lindane and malathion, for example, have been shown to increase microsomal enzyme activity in humans using antipyrine clearance as a marker (Hosler et al., 1979; Uppal et al., 1982). Parathion, on the other hand, has been reported to inactivate the microsomal cytochrome P-450-dependent monooxygenase system (Kulkarni and Hodgson, 1984). The rate of metabolic clearance can be expressed as that of the parent species or individually for metabolites when such clearance processes are first-order (unsaturated) in nature. For example, the pharmacokinetics of chlorpyrifos in male volunteers was studied by modeling the disposition of its principal metabolite, 3,5,6-trichloro-2-pyridinol (3,5,6-TCP) and determining its renal clearance rate to be 4.6 ml/hr/kg for a 0.5 mg/kg oral dose.

The Michaelis-Menten parameters K_m and V_{max} can also be used when the process appears to be saturable at bioavailable exposure dosages encountered. For humans, these parameters may be obtained through *in vitro* studies on human tissues. Using this approach, Smith and coworkers, incubated human lung slices with paraquat to determine V_{max} and K_m for accumulation of paraquat in human lung via a process exhibiting saturation kinetics (Smith et al., 1974). In the absence of experimental data related directly to humans, Michaelis-Menten parameters from animal studies have been used with allometric scaling to postulate V_{max} and K_m. This approach was used by D'Souza and others in their model on ethylene dichloride, in which they approximated these parameters in humans for a saturable pathway involving P-450 oxidation of ethylene dichloride.

Gross body elimination of the parent pesticide, unchanged by biotransformation, can also be expressed by a clearance rate (Cl) or by Michaelis-Menten parameters, depending upon the nature of the excretion. A valuable derivative elimination parameter, the elimination half-time of a pesticide or its metabolite(s) from plasma or from the body can be derived from $0.7Cl/V$. This parameter is appropriate only when the elimination process is first-order at relevant pesticide exposure levels.

A complete toxicokinetic profile of pesticide disposition in humans would optimally include tissue concentrations of pesticide and pesticide derivatives following supervised dosage exposures under experimental conditions. While ethical considerations preclude experimental exposure of humans to highly toxic pesticides and multiple tissue biopsies, apparently safe dosages of some pesticides have been studied in human volunteers, as evidenced by the examples presented here.

For those studies which do exist, the parameters developed allow for a limited simulation of the time course of certain pesticides following oral ingestion or topical application. However, valuable as they are, they fall far short of serving as a sound basis for risk assessments under a variety of conditions, especially since links between plasma concentrations and toxic effects are lacking in most cases. For example, although plateau concentrations have been estimated in some cases, human pharmacokinetic data on the chronic effects of low-level exposure on enzyme induction or inhibition in metabolic pathways remain largely qualitative or unavailable. In a related issue, much of the classical pesticide work has been on healthy human male volunteers, leaving unanswered questions about relevancy to health effects with variation in age and sex, fluctuations in body composition, state of health, and concomitant drug or xenobiotic exposure.

This fact is important when considering the disposition of pesticides in special groups such as pregnant women, the aged, children, and persons taking therapeutic drugs. The complexity of such special situations is illustrated through a case reported by Branch and Jacqz (1986). They postulated that long-term, household exposure to excessive amounts of carbaryl accounted for subacute neurotoxicity in an elderly male receiving cimetidine, whose wife and son did not show comparable disability. However, the subject's age, state of health, and concomitant therapy with a known metabolic depressant confound interpretation.

Some investigators have directly examined age-associated effects with pesticides. Recent studies in developing rats showed that lindane metabolism and excretion parallel development of hepatic enzymes (Copeland et al., 1986). Moreover, percutaneous absorption studies with rats on 14 pesticides also have shown age and dose-related differences in dermal penetration (Shah et al., 1987).

Only a modest amount of information is available on the disposition of topically applied pesticides in humans, and that which is available varies from the results of well-controlled studies conducted in situations unrepresentative of actual exposures, to results from studies under conditions of use which yield only limited amounts of reliable information. A study on the absorption of lindane in infants and children for whom the chemical had been prescribed to treat or prevent scabies illustrates some of the difficulties associated with acquiring human data. In this study, Ginsburg and coworkers found that in children treated topically with 1% lindane lotion, blood concentrations were independent of quantity absorbed, but inversely related to weight and body surface area. (Ginsburg et al., 1977). In this experiment, however, there was an uncertain dosage applied, and no urine samples collected for analysis, so that the total bioavailable quantity absorbed was undocumented.

Results from these animal and human studies--as well as the generally recognized importance of age in drug disposition and pharmacologic effects (e.g., Mannering, 1985; Mayersohn, 1986; Milsap and Szefler, 1986)--all point to the need (1) for caution in applying animal and limited human pharmacokinetic data to special situations; and (2) for acquiring better disposition data over a range of biologic conditions.

Recent efforts at physiologically-based pharmacokinetic modeling are attempting to address some of these time-related concerns. Yesair and colleagues have modelled the absorption, distribution, and elimination of hexachlorobenzene (HCB) in growing humans. Using a model based on the rat, they forecast the disposition of HCB in a 15-year-old human female simulated to have a 15-week exposure to daily doses of 0.5 to 32 mg/kg HCB. Their results gave an estimated elimination half-life of 215 days, which they interpreted to mean that a four-year monitoring program would be needed to assess human exposure properly. Their model for the pregnant human also estimated extensive transfer of hexachlorobenzene via milk to the nursing offspring (Yesair et al., 1986). Systematic, educated forecasting paradigms such as this are useful for exposing the many assumptions that currently must be made in applying rodent disposition data to humans.

In other work, Reitz et al. (1987) have used physiologically-based pharmacokinetic models in attempts to predict the effect of aging upon the disposition of methylene chloride in aging rats. They observed that some investigators have found it necessary to increase the relative percentage of fat when constructing pharmacokinetic models for older animals. In their work, they increased the fat compartment from 7.08% to 28.3% of total body weight and found that the model compared favorably with actual data on nine parameters. They noted, however, that similar success was not observed in the work of Schumann on geriatric mice, and suggested that the disposition of methylene chloride in older mice might involve factors other than body fat.

Andersen (1987) emphasized the influence that time-dependent physiologic and metabolic changes may have on the kinetic behavior of xenobiotics, and observed that--in theory--physiologic models allow for these influences through specific definitions of metabolic constants and physiologic parameters for organs of elimination and target tissues. He noted the potential value of studies on biochemical and physiologic parameters at the end of chronic toxicity studies and also urged better understanding of metabolic capabilities in non-hepatic tissues which may affect presystemic elimination. Andersen suggested that physiologically based pharmacokinetic models--which ideally should allow for dose, route, and species extrapolations among studies--may provide a means for using the limited data from inhalation studies to evaluate the risks of consuming drinking water contaminants.

Another major gap in existing human pharmacokinetic data is general lack of relevance to actual exposure conditions--a consideration especially important when evaluating occupational health effects of pesticides wherein dermal or inhalation exposure, the extent of exertion, humidity, mode of covering, and exposure schedule all may play significant roles in pesticide disposition.

More exhaustive studies are needed to elucidate the application of principles of dermal penetration under actual conditions of pesticide exposure. For example, although the sweat glands themselves may affect absorption and excretion minimally, the secretion of sweat and the extent of occlusion influencing sweat retention could alter the absorption of pesticides. Through simple washing, sweat may decrease absorption, or by influencing hydration of the stratum corneum, sweat may enhance absorption of water-soluble compounds and decrease absorption of lipophilic compounds.

The paucity of human data and the ethical restraints upon experimentally obtaining such information underscore the need for prudent use of accidental exposures (where possible) to study metabolic changes, as well as chemical concentrations in blood, urine, or other tissues and their possible relationships to observed toxic effects. Similarly, well-designed investigations into occupational exposures may provide insights into relationships between chronic exposures and effects.

Biologic monitoring for occupational exposures to pesticides has been limited to only a few chemicals, including pentachlorophenol, methyl bromide, and chlordimeform. Nevertheless, interest in biologic monitoring is mounting, and is becoming increasingly important in field investigations for assessing and improving the safety of conditions and equipment associated with occupational exposures to pesticides. Recently, Nigg and Stamper have extensively reviewed current practices and their shortcomings, as well as new approaches in biologic monitoring for pesticide exposure. (Nigg and Stamper, 1989). Coye and colleagues also have reviewed analytic methods for detecting numerous pesticides or their metabolites in biologic samples from workers (Coye et al., 1986).

Considerable interest has focused upon monitoring urine to estimate actual "internal doses" of pesticide exposure (Franklin et al., 1981). For example, Morgan and coworkers measured the nitrophenol and alkyl phosphate metabolites in the urine of human volunteers who ingested one to four milligrams of methyl and ethyl parathion. This study reported observing sufficient dose-dependence to suggest coefficients relating parathion absorption to total metabolite excretions (Morgan et al., 1977).

Maddy et al. (1988) used rodent data as a model in monitoring the exposure of strawberry harvesters to captan. In this model, they used information from rat studies to estimate the amount of captan absorbed through skin, and the percent of a captan metabolite, THPI (*cis*-1,2-dicarboxyimido-4-cyclohexene), excreted in the urine. They reported that estimated captan exposure ranged from 96 mg/day using a foliar residue method to 0.2 mg/day using biologic monitoring, dramatically illustrating the influence of monitoring methods upon exposure estimates.

To be useful, biologic monitoring must be based upon more than simple qualitative detection of the presence or absence of a chemical in a biologic sample. To be quantitative, it must be founded upon an established relationship between the rate of excretion of the monitored substance at the anatomic collection site (e.g., urine, hair, or saliva), and either (a) the concentration of the substance in the circulation; or (b) the total amount of the substance in the body. Additionally, when inactive metabolites are measured, it must be established that formation of the metabolite occurred within the body and not prior to or during the absorption process if the first-pass effect or extent of bioavailability of the parent pesticide is to be taken into account.

To make some judgment about the safety of exposure to the bioavailable "internal dose," some established correlation is needed relating this "internal dose" of the substance to biologic toxic effects. If the safety judgment is based upon animal experimental data relating dose to toxicity, and the route of exposure for that experiment is different from the human exposure route of concern, then the true bioavailability of the substance via the experimental route must also be known, and not merely the gross, external dose of the experiment.

Practical difficulties in obtaining human data through traditional means, such as blood and urine analyses, also point to the need for more and better non-invasive, epidemiologically based methods of exposure assessment. Under standardized conditions, for example, concentrations of xenobiotics in saliva may track free concentrations in blood, although saliva volume is difficult to quantify. (Evans et al., 1986). Saliva analyses, as well as hair, nail, and breath analyses currently are underdeveloped methods showing promise as non-invasive routes to exposure assessments (Ishiyama et al., 1983; Johnson et al., 1971; Suzuki et al., 1984). Additionally, development of new technologies, such as one measuring outward transcutaneous chemical migration, represents further efforts to sample chemicals from humans non-invasively (Peck et al., 1988).

In all cases, however, it is critical that chemical and tissue measurements be embedded in a richer data base encompassing time-integrated monitoring information and gross exposure estimates. Recognition of this fact will be especially important as greater attention focuses on understanding the composition of foods, including natural toxicants, and how food consumption patterns may influence biologic effects and pharmacokinetic parameters. Indeed, given that chemical disposition patterns are often modified by the co-administration of other substances, it may be necessary to re-establish pharmacokinetic data if developments in food technology lead to significant compositional changes in food matrices, or behavioral patterns significantly alter consumption patterns.

Most pesticide exposure to applicators and to other persons who subsequently contact the residues, including children, is by the dermal route. The amount of material absorbed via the dermal route depends on the area of skin exposed, the condition of the skin (hot, moist, etc.) and the nature of the pesticide formulation: pesticides in liquid formulations are more readily absorbed than those in dusts or granular form. The use of a solvent, such as xylene, accelerates the absorption rate and affects the physicochemical nature of certain chemicals used as active ingredients.

Some chemicals with less than a 1% dermal absorption rate in 24 hours allow for most use of certain very toxic chemicals with an average amount of protective clothing and equipment, while the nearly 90% dermal absorption rates in 24 hours of nitrophenol ether and chlordimeform make these chemicals almost impossible to use with any degree of safety.

Very few pharmacokinetic data in humans exist for chemicals used as pesticides. Lacking such information, one can extrapolate from metabolism studies conducted in test animals and assume that similar levels of parent chemical or metabolites can be found in blood or urine of individuals exposed to similar levels.

If these data are not available, skin absorption rate studies can be conducted in rodents, monkeys or humans. Radiotracer technology can be used in animals. Dermal dose-response studies can be conducted on cholinesterase inhibitors in humans and animals; however, other more toxic end points must not be overlooked.

For any given exposure situation being studied, the dermal dose can be measured by skin washing, solvent extraction of full-body underwear, extraction chemical from cloth pads placed on the skin and or outer clothing. Potential inhalation dose can be measured with appropriate air sampling pumps and filters. The hours, days per year, and years of exposure in a lifetime need to be estimated for both the typical and the most exposed workers. The final step in risk assessment involves a comparison of the exposure estimate in relation to the no-observed-effect level (NOEL) or carcinogenic potential of the pesticide. The problem of incorporating short-term or intermittent exposures into carcinogen risk assessment has not yet been resolved.

OCCUPATIONAL EXPOSURE TO PESTICIDES

The occupationally exposed population consists of pesticide mixers, loaders, applicators, and field workers (see Table 2-1). There are many exposure studies in the scientific literature on this population; these include a sizable amount of human data on the exposure of pesticide applicators to specific pesticides. Workers are generally exposed dermally to quantities of pesticides amounting to several mg/hr, depending on work practice and application rate.

Forest workers applying 2,4,5-T were monitored to determine the amount of pesticide in the breathing zone, the amount deposited on patches attached to clothing and the amount excreted in the urine of exposed workers. Ranking of exposure level by worker duty revealed that concentrate mixers and backpack applicators had a higher absorbed dose, as measured by urinary excretion, than did mist blower operators, helicopter pilots, supervisors, or helicopter flagmen. Supervisors and helicopter flagmen received lower exposure than others in the study (Lavy, 1978; Lavy et al., 1987). Aerial application crews were found to have considerably lower levels of 2,4,5-T exposure than ground crews (Lavy et al., 1982). Personal work habits and attitude are important criteria in determining pesticide exposure. Backpack applicators were the most heavily exposed of 80 workers applying phenoxy herbicides and picloram, using group application techniques (Lavy et al., 1987). Exposure was highest in mixers who handled the spray concentrate and in sprayers using backpack equipment. Some absorption of 2,4,5-T was apparently due to wearing contaminated clothing by field workers (Leng et al.,

TABLE 2-1. Estimated Numbers of Workers Exposed to Pesticides in the U.S.A.

Workers	Sites/Sources	Type of Pesticides	Estimated Numbers
Janitorial service	Buildings	Disinfectants, insecticides, fungicides, ralendicides	Several million
Plant growers and harvesters	Greenhouses, vegetable, vine and tree crops	Variety	Several million
Farmers	Crops, livestock	Variety	2.17 million
Professional ground applicators	Farms, parks, roadsides, other outdoor areas	Variety	200,000
Structural applicators	Factories, food processing plants, hotels, offices, homes, apartments, other structures	Fumigants, termiticides, other pesticides	150,000
Mixer loaders, flaggers, clean-up and repair staff	Crops, mosquito control	Variety	20,000
Aerial applicators	Crops, mosquito control	Variety	7,000

1982). Libich et al. (1984), working with phenoxy herbicides in Canada, also showed much higher dermal absorption than via inhalation pathways.

The occupational exposure of commercial lawn specialists applying 2,4-D was below the WHO/FAO acceptable daily intake of the compound (Yeary, 1986). Mixers of pesticide concentrates and other applicators who become wetted with the spray solution receive much higher doses than others walking through herbicide-treated areas (Lavy, 1987).

A large body of data involving air level measurements in the breathing zone and skin contamination measurements, as well as metabolism and skin absorption study data on specific pesticides now is provided to regulatory agencies; nonetheless, there is still a major need for internal dose data collected on humans.

Data on inhalation and dermal exposures to specific pesticides are also in registration standards and special review documents published by EPA, state regulatory agencies, and regulatory agencies in other countries. These documents provide actual airborne levels--amounts of pesticides that are deposited on the skin--for a particular type of work practice, the dermal absorption rate in test animals, and metabolic data that have been developed, (also usually in test animals). The great complexity of patterns of use, and thus the great diversity of opportunities for exposure, are outlined on pages 87 to 92 of Title 40 of the Code of Federal Regulations. Efforts to collect information on worker exposure within pesticide manufacturing plants have been hindered by company "confidentiality" policies. Some companies have collected considerable information to comply with OSHA guidelines, but this information is not publicly available. In contrast, the lawn care industry continues to study employee and client exposure to pesticides. Segments of this industry have kept excellent records of employee contact hours, specific pesticides, and health records for the past 15 years.

Exposure measurements on field workers have been reported in a number of scientific publications and in reports developed by pesticide registrants for regulatory agencies and in recent symposia sponsored by the American Chemical Society (Honeycutt et al., 1985; Plimmer, 1982; and Wang et al., 1989). Table 2-2 identifies occupational pesticide exposure categories; and Table 2-3 provides information on field worker exposure to pesticides.

TABLE 2-2. Potential Occupational Pesticide Exposure[a]

High	Medium	Low
Mixers, loaders	Applicators	Manufacturing
Applicators	Groundrig (open cab)	Mixer, loader
Groundrig (airblast)	Groundrig (row)	Closed system
Backpack sprayer	Flagger	Applicators
Structural-PCO	Fumigators	Aerial
Lawn and garden	Greenhouse	Groundrig (closed cab)
Right-of-way	Field	Groundrig (granule)
Flaggers	Orchard, grove,	Groundrig (wick)
Fumigators	vineyard	Fieldworkers
Pet shampooers/		least foliage in row
dippers		or low crops, e.g.
(commercial)		pickles, cabbage,
Animal dippers		sweetcorn
		Mushroom workers
		Hospital workers
		Forestry workers
		Transportation workers

[a] Personal communication: M. Moses and J. McCarthy. The three exposure categories listed (high, medium and low) are only intended to approximate relative levels of exposure. Clearly, exposures in all categories will be dependent on many factors, such as work practices, protective measures, and level of training.

NOTE: Fieldworkers, commercial applicators, and private applicators may be certified or work under certified applicator supervision.

TABLE 2-3. Fieldworker Exposure to Pesticides[a]

States with Highest Fieldworker Exposure	Major Crops with High to Moderate Fieldworker Exposure	Major Crops with Minimal Fieldworker Exposure
California	Citrus	Corn
Texas	Apples	Soybean
Florida	Tomatoes	Cotton
Midwestern States	Strawberries	Wheat
	Vegetables	
	Grapes	
	Tree crops	

[a] Personal communication: M. Moses and J. McCarthy. Listings are based primarily on Labor intensity.

EXPOSURE OF THE GENERAL POPULATION TO PESTICIDES

Dietary Exposure to Pesticides

The world's food supplies have changed in many ways over the last several decades, resulting in a greater volume of food for many nations, wider distributions of food varieties, vast arrays of food forms presented by the food technologists, and less occurrence of the many food-borne pests and diseases that frustrated former attempts at storage and distribution. To achieve these beneficial changes, however, the use of pesticides has increased and the introduction of food additives and contaminants during food storage and processing has become a public issue. Food-borne chemicals are now perceived by some to pose a new health problem that varies with the types of chemicals involved, the amount and type of food consumed, and the age and ethnic characteristics of the consumer.

Regulation of pesticide residues in food is a complex task and one which relies heavily on data bases for the chemical characteristics and the toxicologic profile of the pesticide, the effects of storage and processing on the formulation, the consumption profile of the food and its many forms, market trends of import/export, by-product use for animal feed and extent of use of the chemical. Many of these parameters are unique to a given chemical and may be unique to a given nation or geographical region. Efforts to estimate health risks posed by the substance in crops will consequently be more difficult for a wide international population than for a homogeneous group in a small area.

Fortunately, today's scientific technology has provided tools and information bases to address the new problems introduced by this issue. Computer aides make the international use of consumption surveys and dietary studies relatively easy, and information exists for many of the chemicals of concern.

Recognizing the need for more careful and thoughtful analysis of this issue, national and international groups are developing the principles and criteria for evaluations. This is an important task, worthy of assistance from professionals with access to current data, models, and experience.

Standards for exposure assessment and risk assessment for any population or subpopulation should be based not only on the traditional toxicologic parameters derived from chronic exposure studies, but also on the long-term health effects that can result from short-term (acute) exposure. Teratogens and some neurotoxicants, among others, exert their biologic damage after short durations of exposure, but the health effects can be lasting and serious.

Likewise, any exposure estimates that reflect "annualized" estimates of exposure and ignore the estimates of acute exposure will dramatically underestimate the potential of a given dietary pattern to harbor significant chemical exposure levels. For example, the annualized consumption of pineapple in Finland (total annual consumption per person/365 days) is quite small. This annualized consumption figure is valid when compared to toxicologic parameters resulting from chronic exposure studies (e.g., oncogenicity studies) but quite worthless for assessing the potential hazard if the chemical in question is a teratogen. In such a case one needs to know "How much pineapple will be consumed on days when it is actually eaten?" This amount will be much higher. The total consumption of pineapple may be eaten on only one day of the 365 days per year. If it is eaten on a critical day during gestation, along with other commodities bearing the same chemical, the total exposure could be significant.

Risk assessment schemes in international deliberations seem to completely overlook this concept, and consequently the traditional use of acceptable daily intakes (ADIs) and other chronic-exposure health effect parameters are inadequate for international guideline presentations. The U.S. EPA has utilized the acute dietary assessment models successfully, and adoption of these models for all countries is quite feasible.

Water is a major part of the diet and can contain significant pesticide residues or water purification byproducts. The contribution of water-borne residues to overall dietary exposure can be factored into the total dietary exposure profile just as any other dietary commodity is included. For some nations, waterborne residues are a major concern and would influence the acceptability of additional exposure via other components of the diet.

The extent and sophistication of national food processing practices and technologies must be included in risk assessment. Information generally available from the food technology industry on the effects of common processing practices on an individual chemical can inhance the data base to improve the mechanics of exposure estimation; in addition, data on nationally unique processing practices presently exist and can be compiled to formulate a working set of principles to be utilized in risk assessment. The assumption that use of processing data or refined consumption data would only *reduce* projected exposure is not necessarily true. In fact, pesticide residue migration into fractions of the food commodity with high consumption versus into feed constituents or into commodity constituents with lower consumption values would yield an overall *higher* exposure for some populations. In addition, processing procedures which convert a chemical to a more active form must be recognized as increasing the overall risk rather than reducing it. Of particular importance here will be chemical migration into sugars, oils and grain subunits which will constitute significant dietary consumption contributions to some national groups.

These considerations must be founded on actual data and reliable information. Using generalized assumptions can result in dramatic overestimation or underestimation of risk for any given national group.

General Population Survey Data. Two of the major sources of information on pesticide exposure of the general population are the second National Health and Nutrition Examination Survey (NHANES II), conducted during 1976-1980, and the National Human Adipose Tissue Survey (NHATS), conducted since 1970. In NHANES II, the National Center for Health Statistics (NCHS) and the EPA National Human Monitoring Program for Pesticides cooperatively collected and analyzed blood and urine specimens for 38 selected pesticide residues and their metabolites. In the National Human Adipose Tissue Survey, samples collected by cooperating pathologists in geographically dispersed sites across the United States are analyzed (Murphy and Harvey, 1985).

The NHANES II sample was a complex, multi-stage, probability sample of the civilian, noninstitutionalized population of the United States, ages 6 months to 74 years. A subsample of individuals 12 to 74 years old had blood and urine samples collected and analyzed for pesticide residues and metabolites. Since an interfering serum separation substance was present in the vacutainers used for blood collection for several of the Western region samples, weighted estimates are available only for the combined Northeast, Midwest, and South regions of the United States. The NHANES II estimates presented in Table 2-4 for the pesticide residues and metabolites found in blood are weighted to represent the general U.S. population, ages 12 to 74 years, residing in the U.S. Northeast, Midwest, and South during 1976-1980.

Residues of pesticides and their metabolites in various human tissues and fluids are indicative of the total body burden of these pesticides and of past and present exposure to them. As shown in Table 2-4, NHANES II and the NHATS (1979) results indicate that the general population was broadly exposed to selected persistent halogenated hydrocarbons (Murphy and Harvey, 1985).

The difference in the percent exposed (percent with quantifiable amounts) as estimated by NHANES II and NHATS (1979) is expected since the body materials tested were different. Because halogenated hydrocarbons are known to accumulate in fatty tissues, the NHATS estimates are indicative of any past exposure.

A more detailed, unweighted analysis of the NHANES II blood data found that older persons, farm residents, and males were more likely to be found to have quantifiable amounts of these selected halogenated hydrocarbons in their blood (P. Stehr-Green, Medical Epidemiology, Division of Immunization, Centers for Disease Control, Atlanta, Ga., personal communication, 1988).

The most frequently occurring residue found in the NHANES II urine samples was pentachlorophenol (PCP), found in quantifiable levels in about 72% of the general population (F. W. Kutz, Modeling and Monitoring

Systems, U.S. Environmental Protection Agency, Washington, D.C., personal communication, 1988). Percent quantifiable levels of PCP were found to be highest among males and blacks. Quantifiable levels were also found of 3,5,6-trichloro-2-pyridinol (5.8%), dicamba (<2%), and malathion monocarboxylic acid and dicarboxylic acid (<2%).

TABLE 2-4. Percent of Blood and Adipose Tissue Samples Containing Quantifiable Amounts of Pesticide Metabolites and Residues[a]

Pesticide Metabolites and Residues	NHANES II[b] (1976-1980)	NHATS (1979)
p,p'DDT	31%	98.6%
p,p'DDE	99%	99.1%
β-Benzenehexachloride	13.9%	92%
Dieldrin	8.6%	96%
Trans-Nonachlor	4.4%	97%
Hexachlorobenzene	3.3%	91%
Heptachlor Epoxide	2.5%[c]	95%
Oxychlordane		94%

[a] From Murphy and Harvey, 1985.
[b] Weighted to represent 12 to 74 years old U.S. population residing in the Northeast, Midwest, and South from 1976 to 1980.
[c] Quantifiable amounts of Heptachlor Epoxide and/or Oxychlordane.

In addition to measuring residues of active pesticide ingredients, it is likely that increasing attention will be given to determining the possible presence of many of the so-called "inert" ingredients of pesticide formulations. It is apparent that there is a large exposure potential to more than 1,000 inert chemicals and that these materials deserve more attention than they have received in the past.

Up-to-date estimates of body burdens of the general U.S. population will not be available for several years, when the NHANES III (now ongoing) volatile toxicant subsample data have been collected and analyzed. The Hispanic Health and Nutrition Examination Survey (HHANES), conducted 1982 to 1984 by NCHS, collected blood and urine samples on the three largest Hispanic groups in the United States: Mexican-Americans in the Southwest, Cubans in Dade County, Florida, and Puerto Ricans in the New York City area. EPA has analyzed the samples, and the data are now being edited. Many Mexican-Americans living in the Southwest United States are likely to have had occupational pesticide exposure. Preliminary analysis of HHANES data found that 2% of 12 to 74 years old Mexican-Americans in this time period were migrant farmworkers at the time of the survey, 7% had been migrant farmworkers in the past, and 20% were, or had been in the past, non-migrant farmworkers (Brody et al., 1988).

Easy-to-use computer programs for consumption data and exposure analyses are available for geographically specific populations of other countries and individual agencies within those countries. New data about consumption estimates unique to the individual countries can be used to modify existing national data bases or to construct *de novo* consumption files. Programs for the use of that information or for the use of auxiliary data have been developed. These systems are inexpensive and can be utilized by health professionals with little or no previous computer experience. Rather sophisticated exposure analyses and exposure distributional analyses can be generated for chemicals under consideration to demonstrate the probable risk presented to different geographical populations. Relatively few assumptions need to be made in conducting these analyses, as data on the variables of exposure are becoming more available.

Exposure to Naturally Occurring Toxicants. It has been estimated by Doll and Peto (1981) that as much as 35% of all cancer might be related to diet; therefore, it follows that the naturally occurring chemicals in foods may be the cause. Potential naturally occurring toxicant levels in foods have been estimated to be 10,000 times that of the synthetic pesticides in the same foods (Ames, 1983). Duke (1989) has shown that in some herbs the value approaches 100,000 times.

Every living thing has some means of survival. Without means of defense, plants would become extinct; and the production of toxic chemicals is a major mechanism of plant defense. Bell (1974) reviewed ways in which plants may express resistance to pathogens. A plant's ability to modulate levels of toxic chemicals in response to tissue damage or other external stimuli is a phytoalexin response, and these toxic chemicals are referred to as phytoalexins. Some of these chemicals are the coumarins, glycolalkaloids, isocoumarins, isoflavonoids, linear furanocoumarins, stilbenes, and terpenoids. Phytoalexin chemicals found in common food plants are shown in Table 2-5. We often use plant chemicals as templates for synthetic pesticides. It is fitting and correct to refer to them as naturally occurring pesticides, or natural pesticides.

The best understood plant-toxin system for plant-produced chemicals with resultant human toxicities is the celery plant (Beier, 1989). Celery contains at least three photoactive linear furanocoumarins: bergapten, psoralen, and xanthotoxin (Beier et al., 1983b). These compounds are phytoalexins in celery (Beier and Oertli, 1983) and were initially thought to be produced by only diseased celery (Wu et al., 1972), but they are easily observed in healthy, normal appearing celery at a combined level of about 1 $\mu g/g$ (Beier et al., 1983).

These natural pesticides are potent photosensitizing compounds and can express biologic activity in a broad spectrum of living things, from fungi to humans. The diverse activity of linear furanocoumarins is due to intercalation into DNA and RNA where they can form covalent bonds in the presence of long-wave U.V. light resulting in cross-linked DNA. They may be mutagenic (Ashwood-Smith et al., 1986), and have displayed photocarcinogenicity during medicinal use (Stern et al., 1979). Linear furanocoumarins also can cause cataract formation in both animals and humans (Lerman, 1986).

Centers for Disease Control (CDC) studied outbreaks of phytophotodermatitis among grocery store workers in 1984 (Berkley et al., 1986) and in 1986 (Seligman et al., 1987), and have shown that one particular new brand of celery was responsible. This particular cultivar was produced because it had more insect resistance, and it also had a total linear furanocoumarin level in the stalks of 8-10 $\mu g/g$ or 10 times the levels previously observed in celery (Beier et al., 1983b).

A thorough treatment of the following toxicants and others are found in the review on "Natural Pesticides and Bioactive Components in Foods" (Beier, 1989).

Crucifers, including broccoli, brussels sprouts, cabbage, and cauliflower contain the known goitrogens, thiocyanate, goitrin, and allyl isothiocyanate. These have recently been shown to modulate carcinogenicity (Beier, 1989). When crucifers are given prior to a carcinogen, they protect against carcinogenesis; whereas, when crucifers are given after a carcinogen, they strongly promote carcinogenesis. This may be similar to 2,3,7,8-tetrachloro-

TABLE 2-5. Phytoalexins in Some Food Plants

Plant	Phytoalexins
Alfalfa	vesitol, sativan, medicarpin
Pea	pisatin, cinnamylphenols, 2'-methoxychalcone
Soybean	glyceollin
Bean	phaseollin
Broadbean	wyerone
Grapes	viniferin from resveratrol
Cotton	gossypol, cadalenes, lacinilenes, hemigossypol
Peanut	resveratrol
Celery Parsley	furanocoumarins
Parsnip	furanocoumarins
Rice	momilactones, oryzalexins
Castor bean	casbene
Potato	rishitin, hydroxylubimin, phytuberin, a-solanine, a-chaconine, lubimin, solavetivone, phytuberol
Pepper	capsidiol
Sweet potato	ipomeamarone
Carrot	6-methoxymellein, falcarinol
Tomato	alpha-tomatine, rishitin, falcarindiol, falcarinol
Lima bean	5-deoxykievitol, 8,2'-dihydroxygenistein
Tobacco	rishitin, lubimin, phytuberin, phytuberol, solavetivone, capsidiol, glutinosone
Eggplant	lubimin

dibenzo-*p*-dioxin (TCDD) exposure. These results are found to be caused by the action of the plant product indole-3-carbinol (I3C). I3C condenses in the stomach acid to produce at least four condensation products (Bradfield and Bjeldanes, 1987); at least three of these bind to the TCDD receptor and compete with TCDD for the TCDD receptor.

Foods in the nightshade family, such as eggplant, garden peppers, tomatoes, and white potatoes, contain cholinesterase inhibiting glycoalkaloids. Factors in this group of vegetables are thought to be important in some arthritis episodes. For example, the average nitrate level in potatoes in 119 μg/g fresh weight, but tubers may contain over 1200 μg/g (Beier, 1989).

The glycoalkaloids in the nightshade family may be possible teratogens. New sprouts of all potato varieties tested (Kennebec, Nampa, Norchip, Pioneer, Russet, Sebago, and Targhee) were teratogenic including spina bifida in Siomesen hamsters (Keeler et al., 1978). Solasodine, a spirosolane alkaloid found in eggplant was found to be embryotoxic and teratogenic in rats (Seifulla and Ryzhova, 1972). Potatoes that contain more than 200 μg/g total glycoalkaloids (TGA) per tuber are considered unfit for consumption (Beier, 1989). The average TGA contents of potato tubers from Lenape, Kennebec, Russet Burbank, Katahdin, Irish Cobbler, and Red Pontiac potato varieties were 293, 97, 79, 79, 62, and 43 μg/g potato, respectively (Sinden and Webb, 1972). These chemicals were not affected by baking, boiling, or microwaving, and were only slightly reduced by frying. The levels of these chemicals in the variety "Lenape" caused its quick withdrawal from the marketplace because of acute toxicity to humans (Jadhav et al., 1981). These glycoalkaloids are phytoalexins and dramatically increase in response to tissue damage, and other external stimili (ex. light, cold, fungicides). As a result of bruising, the variety "Katahdin" had an increase of TGAs to 1720 μg/g at 5C, and 220 μg/g at 20C (Mondy et al., 1987). Livestock have died after ingesting potato vines, green potatoes, or tomato vines. Human poisoning episodes and fatalities have been reported (Beier, 1989).

Mushrooms contain hydrazines that may be carcinogenic after metabolic activation. The hepatotoxic and carcinogenic *N*-methyl-*N*-formylhydrazine (MFH) is more mutagenic after metabolic transformation into *N*-nitroso-*N*-methylformamide (MNFA) by microsomal monooxygenase. However, NMFA is unstable and can spontaneously decompose producing a methyl cation alkylating agent, which can result in the destruction of cytochrome P-450 (Beier, 1989).

Mycotoxins are found on grains and nuts, especially corn, cottonseed, fruits, grains, grain sorghums, and peanuts. They occur in apple juice, bread, peanut butter, and other products resulting from contaminated starting materials. Aflatoxins are hepatotoxic to fish, mammals, and poultry. They attack the immune system. They have been linked to leukemia, liver cancer, mental retardation, and Reye's syndrome. The mycotoxins zearalenone and zearalenal are estrogenic agents, and it is known that estrogenic agents can cause an increase in plasma levels of cholesterol and triglycerides. Fungal species that produce these estrogenic agents may be a contaminant of barley, and as a result these agents could occur in beer or other barley containing products. Some mycotoxins (ochratoxin A and zearalenone) are teratogens. Patulin, a mycotoxin known to occur in apples and barley, is neurotoxic to animals, and can cause edema of the lungs and brain.

The plant species described here are not the only producers of toxic chemicals. A detailed review of these and others are presented elsewhere (Beier, 1989). Most plants probably contain either high concentrations of toxic chemicals and/or produce phytoalexins in response to disease, foreign chemicals, or injury. Table 2-6 shows the LD_{50} for many natural chemicals.

There has yet to be a risk assessment study of naturally occurring toxicants (except aflatoxin) in foods. About half (20/42) of the few plant toxins that have been tested in animal cancer bioassays are carcinogenic (Ames and Gold, 1989), which is comparable to the proportion of synthetic chemicals carcinogenic in animals (B. N. Ames, personal communication). The National Research Council Committee on Food Protection (NRC, 1973) suggested that plant breeders attempting to develop higher yielding or disease-resistant crops must be alerted to the possible production of undesirable components. That suspicion has now become reality in at least two plant species, celery and potatoes. The toxins in a typical Thanksgiving meal are presented in Table 2-7. Rodricks (1978) states that "we remain abysmally ignorant of the chemical and toxicologic properties of most natural chemicals to which humans have a lifetime of exposure." It is also clear that new approaches are needed for the identification of health hazards presented to humans by naturally-occurring pesticides in foods.

An area of present interest should be the genetic engineering of new plant varieties that contain larger quantities of toxicants. Increasing the toxicants in a crop may prove to be dangerous, and hinder the progress of genetic engineering. However, the genetic engineering of aflatoxin inhibitors into crops like corn and peanuts, protecting them from fungal growth or mycotoxin production, might be a practical and attainable goal.

TABLE 2-6. Toxicities of Representative Toxins in Major Human Food Plants[a]

Toxin	Toxicity				Plant Source
Hydrocyanic acid	oral hmn	LD$_{Lo}$[b]	570	mg	Onion, leek, beet, char, cabbage, broccoli, cauliflower, turnip, orange, tangerine, grapefruit, cucumber, soybean, tobacco, bean, potato, corn
	oral mus	LD$_{50}$[c]	3,700	mg	
	oral dog	LD$_{Lo}$	4	mg	
Hydroquinone	oral rat	LD$_{50}$	370	mg	Plum, pear
	ipr rat	LD$_{50}$	170	mg	
	oral mus	LD$_{50}$	400	mg	
Pyrocatechol	oral rat	LD$_{50}$	3,890	mg	Onion, leek, beet, char, orange, tangerine, grapefruit
	oral rabbit	LD$_{Lo}$	1,000	mg	
	scu rat	LD$_{Lo}$	300	mg	
Acetaldehyde	oral rat	LD$_{50}$	1,930	mg	Beet, char, cabbage, broccoli, cauliflower, turnip, orange, tangerine, grapefruit, tomato, tobacco
Oxalic acid	oral hmn	LD$_{Lo}$	700	mg	Onion,leek, beet, char, cabbage, broccoli, cauliflower, turnip, orange, tangerine, grapefruit, fig, soybean, tomato, tobacco, bean, potato, corn
	oral dog	LD$_{Lo}$	1,000	mg	
Saponin	oral mus	LD$_{Lo}$	3,000	mg	Onion, leek, beet, char, orange, tangerine, grapefruit, cucumber, fig, soybean, tobacco, bean
	scu mus	LD$_{Lo}$	800	mg	
	ivn cat	LD$_{Lo}$	46	mg	
	ivn rabbit	LD$_{Lo}$	40	mg	

TABLE 2-6. Toxicities of Representative Toxins in Major Human Food Plants[a] (Continued)

Toxin	Toxicity			Plant Source
Quercitin	oral rat	LD_{50}	161 mg	Onion, leek, corn
	oral mus	LD_{50}	159 mg	
Quercetin	ipr mus	LD_{Lo}	200 mg	Orange, tangerine, grapefruit, tomato, tobacco
Rutin	ivn mus	LD_{50}	950 mg	Cabbage, broccoli, cauliflower, turnip, fig, tomato, tobacco, bean, potato
Carvacrol	oral rat	LD_{50}	810 mg	Corn
	oral rabbit	LD_{Lo}	100 mg	
Betaine	ivn mus	LD_{50}	830 mg	Beet, char, soybean
Capric acid (decanoic acid)	ivn mus	LD_{50}	129 mg	Orange, tangerine, grapefruit
Caprylic acid (octanoic acid)	ivn mus	LD_{50}	600 mg	Orange, tangerine, grapefruit
Furfural	oral rat	LD_{50}	127 mg	Orange, tangerine,
	oral rabbit	LD_{50}	928 mg	grapefruit, corn
Guanidine	oral rabbit	LD_{Lo}	500 mg	Beet, char, soybean, corn
Histamine tangerine,	scu gpg	LD_{50}	0.5 mg	Beet, char, orange,
	ivn dog	LD_{Lo}	50 mg	grapefruit, tomato
	scu rat	LD_{Lo}	250 mg	
Narcotine	ipr rat	LD_{50}	750 mg	Cabbage, broccoli, cauliflower, turnip, orange, tangerine, grapefruit, tomato, potato

TABLE 2-6. Toxicities of Representative Toxins in Major Human Food Plants[a] (Continued)

Toxin	Toxicity			Plant Source
Nicotine	oral hmn	LD_{Lo}	1 mg	Tobacco
	oral rat	LD_{50}	53 mg	
	oral dog	LD_{50}	9.2 mg	
Solanine	ipr mus	LD_{50}	32 mg	Tomato, potato
	ivn rabbit	LD_{Lo}	20 mg	
Sparteine	scu mus	LD_{Lo}	120 mg	Potato
	ivn rabbit	LD_{Lo}	30 mg	
Trimethylamine	ipr mus	LD_{Lo}	75 mg	Beet, char, tobacco
	ivn mus	LD_{50}	90 mg	
	ivn rabbit	LD_{Lo}	400 mg	
Tylocrebrine	oral rat	LD_{50}	65 mg	Fig
	ivn rat	LD_{50}	32 mg	
Theobromine	oral cat	LD_{50}	200 mg	Coffee
	oral hmn	TD_{LO}[d]	26 mg	

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[a] Duke, 1977; all entries are expressed as per kg body weight.

[b] LD_{Lo} = Lowest published lethal dose.

[c] LD_{50} for rats; the synthetic pyrethroid insecticide allethrin, is 680 mg/kg; the broadly used methylcarbamate insecticide, carbaryl, is 307 mg/kg; and the widely used acaricide, kelthane, is 575 mg/kg.

[d] TD_{LO} = Lowest published toxic dose.

Table 2-7. Thanksgiving Dinner Menu[a]

Course	Chemical Composition Includes
Appetizer	
Cream of Mushroom Soup	Hydrazines
Fresh Vegetable Tray	
Carrots	Carotatoxin, myristicin, isoflavones, nitrate
Radishes	Glucosinolates, nitrate
Cherry Tomatotes	Hydrogen peroxide, nitrate, quercetin glycoside, tomatine
Celery	Nitrate, psoralens
Entree	
Roast Turkey	Heterocyclic amines, malonaldehyde
Bread Stuffing with onions, celery, black pepper, mushrooms	Benzo(*a*)pyrene, di- and tri-sulfides, Ethyl carbamate, furan Derivatives, hydrazines, psoralens, safrole,
Cranberry Sauce	Eugenol, furan derivatives
Choice of Vegetable	
Lima Beans	Cyanogenetic glycosides
Broccoli Spears	Allyl isothiocyanate, glucosinolates, goitrin, nitrate
Baked Potato	Amylase inhibitors, arsenic, chaconine, isoflavones, nitrate, oxalic acid, solanine

Table 2-7. Thanksgiving Dinner Menu[a] (Continued)

Course	Chemical Composition-Includes
Sweet Potato	Cyanogenetic glycosides, furan derivatives, nitrate
Rolls with butter	Amylase inhibitors, benzo(*a*)pyrene, ethyl carbamate, furan derivatives, Diacetyl
Dessert	
Pumpkin Pie with cinnamon and nutmeg	Myristicin, nitrate, safrole
Apple Pie with cinnamon	Acetaldehyde, isoflavones, phlorizin, quercetin glycosides, safrole
Beverages	
Coffee	Benzo(*a*)pyrene, caffeine, chlorogenic acid, hydrogen peroxide, methylglyoxal, tannins
Tea	Benzo(*a*)pyrene, caffeine, quercetin glycosides, tannins
Red Wine	Alcohol, ethyl carbamate, methylglyoxal, tannins, tyramine
Water available upon request	Nitrate
Assorted Nuts	
Mixed Nuts	Aflatoxins

[a] American Council on Science and Health, 1987.

Pesticide Levels in Food

Table 2-8 presents data from the U.S. Food and Drug Administration (FDA) on actual pesticide residues in food. The data presented mainly represent samples of commercial raw fruits and vegetables analyzed by methodology capable of detecting residues of a number of the pesticides subject to the NRC committee's risk estimates. The listing here does not include all pesticides analyzed by FDA.

The FDA has monitored the nation's food supply for pesticide residues since the 1960's. The Total Diet Study is the only U.S. program that measures a broad range of pesticide chemicals in foods *as consumed*. Data from the Total Diet Study, as well as data generated from general food monitoring, have shown that human dietary exposure to pesticide residues is consistently below established safety standards.

Data from years of FDA monitoring of the general food supply have shown that above-tolerance residue violations are rarely found, indicating that pesticide regulations seem to be effective for foodstuffs. Illegal residues can result from misuse, poor agricultural practices, or unusual weather conditions. More commonly, though, violations are the result of applying the pesticide to a crop for which no tolerance has been set.

FDA tests about 14,000 domestic and imported food samples each year and analyzes them for pesticide residues, with emphasis on the raw agricultural commodity. In FY 1987, about 15,000 shipments were sampled. Imported foods constituted more than 50% of the total. Fresh vegetables accounted for the largest number of foods sampled, followed by fruits and dairy products. Illegal residues were detected in about 4% of the samples, and in 57% of the cases, no residue was found. For many of the domestically produced foods consumed in large amounts, no residues were detected in the majority of cases. Of the more than 3,000 fresh vegetable samples analyzed, no residues were detected in 63%; <1% contained residues over tolerance; and about 2% contained residues for which no tolerance had been established. For fresh fruit samples, no residues were found in 50%; <1% contained over-tolerance residues; and <1% contained residues without existing tolerances. The picture for imports was substantially the same, with the exception that the proportion of residues without existing tolerances was somewhat higher, e.g., about 4% for vegetables and 6% for fruits.

No residues were found in 76% of the domestic dairy products (milk, cheese, butter, ice cream, etc.), and none contained violative residues. For imports, 80% of the 270 samples had no residues detected, no residues were

TABLE 2-8. FDA Monitoring of Crops Subject to Tolerances (FY84-87)

Pesticide	Number of Samples	Crops	Crops in Which Residues Formed (number of positive samples)	Residue Range (ppm)	EPA Tolerance (ppm)
Linuron	1,238	Potatoes, wheat, soybeans, carrots, corn	Carrots (21/39)	0.03-0.5	1
EDBC compounds	108	Raw fruits and vegetables	63/108*	15 Samples > tolerance	0.1-60
ETU	96(d)	Raw and processed	37/96	Trace-5.7	
Captan, Captafol, Folpet	2,004(d) 2,347(i)	Tomatoes, oranges, apples	Tomatoes(i) (1 sample)	1.0	15
			Apples(d,i) (163/1,100)	Trace-2.3 (Captan)	25
			Apples(d,i) (3/1,100)	Trace-0.05 (Captafol)	0.25
			Apples(d,i) (35/1,100)	0.01-1.4 (Folpet)	25
Permethrin	4,275(d)	Tomatoes, potatoes, lettuce, apples, soybean, corn	Lettuce (407/2,006)	Trace-8.5	20
	4,482(i)	Tomatoes	Tomatoes (107)	≤ 1.5	2

TABLE 2-8. FDA Monitoring of Crops Subject to Tolerances (FY84-87) (Cont.)

Pesticide	Number of Samples	Crops	Crops in Which Residues Formed (number of positive samples)	Residue Range (ppm)	EPA Tolerance (ppm)
Chlorothalonil	457(d)	Tomatoes	Tomatoes (46)	Trace-1.5	5
	2,058(i)	Tomatoes	Tomatoes (111)	≤ 2.0	5
Benomyl	380(d,i)	Raw and processed	Raw foods (47)	Trace-8.7	5-35
	110	Apples	Apples (11)	Trace-5.9	7
o-Phenylphenol	8(d,i)	Oranges, apples	Oranges (3)	1.0-1.2	10
Acephate	1,850(d) 135(i)	Lettuce	Lettuce(d) (73)	Trace-3.5	10
			Lettuce(i) (2)	0.04-0.12	

*After washing and canning, no EBDC residues were detected.
d = domestic, i = imported

over tolerance, and only 1% contained residues for which no tolerances exist.

The California Department of Food and Agriculture (CDFA) conducts a monitoring program similar in scope to that of the FDA. CDFA data parallel those obtained by FDA in that detectable residues are found in only a small proportion of the treated crops, and the residues found are normally well below regulatory limits, ranging from 1/2 to 1/10 of the tolerances. However, the relation of such factors as average residue level and percent of tolerance to safety is dependent on dose and potency (in the case of carcinogens).

FDA and CDFA also carry out targeted sampling and analysis for pesticides not determined by the commonly used analytical methods. The results obtained from these selective surveys generally parallel those observed in the more routine monitoring. In FY 1987 for example, FDA carried out selective surveys for some highly-publicized pesticides, including EBDCs, daminozide, and aldicarb. No illegal residues were found in any of the commodities sampled.

FDA found during a "total diet" study of 8 population groups during 1982-1984 that pesticide residue levels were well below the tolerances set by EPA (Gunderson, 1988). FDA's monitoring for pesticides in 14,492 samples from October 1, 1986 to September 30, 1987 also showed that the U.S. population exposure to pesticide residues is consistently below established limits. Less than 1% of the samples contained pesticide residues that exceeded regulatory limits (FDA Pesticide Program, 1988).

The Total Diet Study provides a direct measure of dietary intake. Table-ready foods representing the total diet of a variety of American population groups are first analyzed for pesticide residues, and dietary intakes are then calculated. These dietary intakes are compared with acceptable daily intakes (ADIs) established by the Food Agriculture Organization (FAO) of the United Nation's World Health Organization (WHO). ADI is defined as the daily intake of a chemical which, if ingested over a lifetime, appears to be without appreciable risk. Measured dietary intakes of pesticides are consistently below established ADIs, usually by orders of magnitude. Because pesticide residue concentrations in table-ready foods are consistently lower than those found in the raw agricultural commodity, the analytical methods used in the Total Diet Study are modified to permit quantification at levels 5-10 times lower than for general commodity monitoring. Table 2-9 lists dietary intakes for three U.S. population groups as determined in the FY 87 Total Diet Study. Only dieldrin dietary intake exceeds 1% of the ADI.

TABLE 2-9. Pesticide Intakes (μg/kg body wt/day) Found in Total Diet Analyses and Their Acceptable Daily Intakes (ADIs) for 3 Age/Sex Groups

		Age, Sex of Group		
Pesticide	FAO/WHO ADI[a]	6-11 mo.	14-16 yr M[b]	60-65 yr F[b]
Acephate	0.5(T)[c]	0.0025	0.0031	0.0047
Aldoxycarb	5[d]	0.0002	0.0001	0.0002
BHC, alpha + beta	—[e]	0.0019	0.0017	0.0007
BHC, gamma (lindane)	10	0.0010	0.0018	0.0007
Captan	100	0.0194	0.0088	0.0244
Carbaryl	10	0.1550	0.0173	0.0227
Carbofuran, total	10[d]	0.0001	0.0001	0.0001
Chlordane, total	0.5[d]	0.0015	0.0018	0.0015
Chlorobenzilate	20	0.0090	0.0031	0.0026
Chlorothalonil	5(T)	0.0000	0.0000	0.0001
Chlorpropham	—	0.1432	0.2072	0.1189
Chlorpyrifos	10	0.0207	0.0065	0.0040
Chlorpyrifos-methyl	10	0.0054	0.0066	0.0036
2,4-D	300	<0.0001	<0.0001	0.0001
DCPA	—	0.0015	0.0011	0.0019
DDT, total	20[d]	0.0348	0.0191	0.0104
Diazinon	2	0.0146	0.0123	0.0066
Dicloran	30	0.2767	0.0682	0.1793

TABLE 2-9. Pesticide Intakes (μg/kg body wt/day) Found in Total Diet Analyses and Their Acceptable Daily Intakes (ADIs) for 3 Age/Sex Groups (Continued)

		Age, Sex of Group		
Pesticide	FAO/WHO ADI[a]	6-11 mo.	14-16 yr M[b]	60-65 yr F[b]
Dicofol, total	25[d]	0.0329	0.0103	0.0101
Dieldrin	0.1[d]	0.0101	0.0045	0.0040
Dimethoate, total	2[d]	0.0117	0.0020	0.0058
Endosulfan, total	8(T)[d]	0.0724	0.0206	0.0400
Endrin	0.2	<0.0001	<0.0001	0.0001
Ethion	6(T)	0.0168	0.0063	0.0070
Fenitrothion	3	0.0004	0.0005	0.0005
Folpet	10(T)	0.0078	0.0029	0.0096
Fonofos	—	<0.0001	<0.0001	<0.0001
Gardona	—	0.0002	<0.0001	0.0001
Heptachlor, total	0.5[d]	0.0030	0.0018	0.0009
Hexachlorobenzene	—	0.0026	0.0018	0.0010
Iprodione	300	0.0064	0.0025	0.0038
Linuron	—	0.0010	0.0003	0.0004
Malathion	20	0.1395	0.1193	0.0710
Methamidophos	0.6	0.0092	0.0087	0.0215
Methiocarb	1[d]	*t*	0.0004	0.0030

TABLE 2-9. Pesticide Intakes (μg/kg body wt/day) Found in Total Diet Analyses and Their Acceptable Daily Intakes (ADIs) for 3 Age/Sex Groups (Continued)

		Age, Sex of Group		
Pesticide	FAO/WHO ADI[a]	6-11 mo.	14-16 yr M[b]	60-65 yr F[b]
Methomyl	10(T)	0.0022	0.0029	0.0033
Methoxychlor, *p,p'*	100	0.0007	0.0005	0.0003
Mevinphos, total	1.5[d]	0.0126	0.0046	0.0139
Monocrotophos	0.6	<0.0001	<0.0001	<0.0001
Omethoate	0.3[d]	0.0025	0.0011	0.0034
Parathion	5	0.0062	0.0007	0.0016
Parathion-methyl	20	0.0001	0.0001	<0.0001
Pentachlorophenol	—	0.0001	0.0002	0.0001
Permethrin, total	50[d]	0.0710	0.0300	0.0405
Perthane	—	0.0042	0.0016	0.0024
Phosalone	6	0.0808	0.0034	0.0040
Phosmet	20	0.0078	0.0021	0.0049
Pirimiphos-methyl	10	0.0012	0.0016	0.0002
Quintozene, total	7[d]	0.0011	0.0011	0.0005
Sulfur	—	0.0025	0.0009	0.0031
Tecnazene	10	[f]	<0.0001	<0.0001

TABLE 2-9. Pesticide Intakes (μg/kg body wt/day) Found in Total Diet Analyses and Their Acceptable Daily Intakes (ADIs) for 3 Age/Sex Groups (Continued)

		Age, Sex of Group		
Pesticide	FAO/WHO ADI[a]	6-11 mo.	14-16 yr M[b]	60-65 yr F[b]
Toxaphene	—	0.0101	<0.0138	0.0099
Vinclozolin	40(T)	0.0121	0.0044	0.0145

[a] ADIs are usually expressed as mg/kg body wt/day but as expressed here as μg/kg body wt/day for ease of comparison.

[b] M = male, F = female.

[c] "Temporary" ADI.

[d] Includes other (related) chemicals.

[e] ADI not established.

[f] No consumption of food item containing this residue in this age/sex group.

The Total Diet Study methods are capable of determining only about half the pesticides with U.S. food tolerances; however, surveys of selected pesticides indicate that intakes would be similar for pesticides not covered inthe program. These selective surveys show that, when residues are found, the levels are generally small fractions of tolerance values.

Other organizations have reached different conclusions regarding pesticides in food. Their conclusions generally are based on worst-case, *theoretical* estimates of residue levels as well as gross assumptions, since all shipments of all commodities are not continuously analyzed for all pesticides. Concerns about reported detection of persistent organochlorine pesticides are based on unconfirmed data at or near the limits of detection (0.001 ppm). These low-level organochlorine residues generally arise from ubiquitous environmental contamination by these pesticides. Detected levels of pesticides in food are generally well below tolerance levels and substantially below ADIs.

The California Department of Food and Agriculture (CDFA) also utilizes analytical programs to detect pesticide residues on produce. The CDFA Chemistry Laboratory uses specific (single residue) analyses, as well as multiresidue screens for organophosphates, chlorinated hydrocarbons, and *n*-methyl carbamates, and other pesticides. Multiresidue screens monitor pesticides on crops and can quantify pesticide residues well below the tolerance level, within 4-6 hours. Speed allows the CDFA to investigate incidents of illegal residues quickly and, in most cases, to prevent produce containing illegal residues from reaching the consumer.

The multiresidue screen is only one type of detection method. CDFA can analyze virtually all pesticides registered for food crops. Other pesticides, not actually listed as screenable, can be qualitatively identified through the screens. For these chemicals another specific (single residue) method is used to determine the level. [Nine of the 53 pesticides on the EPA list provided to the National Research Council (NRC) fall under this category (NAS 1987). The NRC report, Regulating Pesticides in Food: The Delaney Paradox, identified 55 pesticides as "potentially oncogenic pesticides," by common or trade name. Cypermethrin was listed by active ingredient and two trade names (Ammo and Cymbush). Permethrin was also listed with two trade names (Ambush and Pounce). The list actually contains only 53 pesticides by active ingredient.]

When selecting samples, the CDFA staff request these specific pesticide analyses based on knowledge of pest problems, possible increased pesticide usage, or suspicion of possible pesticide misuse, and identifies pesticides of concern, such as pesticides used in other countries on crops for which they are not registered in the United States. Examples of pesticides targeted for specific analyses include acephate, monitor, omethoate, and permethrin on produce from Mexico, procymidone on grapes from Chile, and cyromazine in eggs.

Although the state does not routinely test for all 53 pesticides listed in the NRC report, CDFA analyses 42 of these pesticides in specific ways:

- Twenty-four can be quantitatively or qualitatively identified through the multiresidue screens.

- Four have been targeted in the Focused Monitoring Program (benomyl, mancozeb, maneb, and zineb).

- Thiophanate methyl is similar in its mode of action and use patterns to benomyl. Both thiophanate methyl and benomyl have the same metabolite, MBC. When the Focused Monitoring analysis of benomyl has been completed, thiophanate may be considered for future Focused Monitoring Programs.

- Seven are not registered in California and have minimal or no food uses elsewhere (calcium arsenate, chlordimeform, copper arsenate, cyromazine, diallate, lead arsenate, and sodium arsenate).
- Three had minimal or no sales in California from 1985 through the third quarter of 1987.
- Four are not being used on fresh market foods (acifluorfen has a California registration for use on soybeans only (a very minor California crop), arsenic acid has a registration for manufacturing purposes and for forest use, asulam is registered for uses on sugarcane (none is grown in California) and alfalfa, and ethylene oxide is used only on a few processed spices).
- Of the remaining 10, paraquat and glyphosate are herbicides that are never applied directly to the crop. Sodium arsenate is applied only to dormant grapevines. For daminozide, the U.S. Food and Drug Administration (USFDA) recently conducted a monitoring program in Washington and Oregon and found that residues detected were far below the established tolerance.
- Three are herbicides which are applied to the soil surface. Oryzalin and methanearsonic acid are not applied directly to the foliage of crops on which they are registered for use; this would result in injury to the growing plants. Metolachlor is not applied to emerged crops. Since metolachlor is used only as a pre-emergent herbicide, it disappears over the growing season and should not present a residue problem at harvest. Metolachlor will, however, be added to the CDFA Focused Monitoring Program.
- Three additional materials have been added to the Focused Monitoring Program (metiram, o-Phenylphenol, maleic hydrazide).

Over the past two years, in the course of its ongoing Pesticide Monitoring Program, CDFA has tested for 9 of the 10 chemicals of greatest concern to the NRC. The only pesticide not tested, chlordimeform, is no longer registered or used in California and has been used only on cotton in the United States during the past 10 years. In 1986, CDFA expanded its program to complement the traditional marketplace surveillance program by developing the Focused Monitoring Program to target nonscreenable chemicals and pesticides of potential health concern.

The CDFA conducts a Pesticide Residue Monitoring Program for produce destined for processing. Between July 1986 and January 1988, 1,777 samples were analyzed using the multiresidue screens. No pesticide residues were detected in over 80% of the samples taken. Approximately 17% of the total samples contained detectable pesticide residues, all within 50% or less of the tolerance. Five processing samples, or 0.28% of the total, contained illegal residues. Of the illegal residues detected, three incidents were the result of drift rather than direct application.

Pesticide use patterns in California show that growers never use every pesticide registered, rarely use pesticides at the maximum label rate, rarely make as many repeat applications as legally permitted, and rarely apply just at the shortest legal preharvest interval. The CDFA Residue Monitoring Program has repeatedly demonstrated that pesticide residues remaining on food are well below the maximum tolerances allowed. In 1987, almost 80% of the 7,010 CDFA routine samples analyzed had no detectable residues. An additional 18% contained residues at levels less than 50% of the tolerance (CDFA, 1988). Approximately 1.5% had illegal levels, and 0.3% of the samples actually had residues in excess of their established tolerance. In 1986 and 1987, 1,297 samples were collected in the Focused Monitoring Program. Samples consisted of commodities known to have been treated with specific pesticides. Approximately 90% of these samples contained no detectable residues of the specific pesticide with which they were treated and for which they were analyzed. The pesticide levels of the remaining 10% were well within the established tolerances.

The following summarizes the CDFA Program activities in 1987. Table 2-10 is a list of 53 potential carcinogens with a description of how they are covered under the CDFA Program.

CDFA Marketplace Samples

- 7,010 samples were taken at wholesale markets, chainstore distribution centers, retail markets, and packing houses.
- A compliance rate of 98.5% of the samples was found in 1987.
  - No residues were detected in 79.8% of the samples.

TABLE 2-10. California Department of Food and Agriculture Activity on Pesticides Considered by EPA as Animal Carcinogens

Pesticide	Activity
Alachlor	On screen
Chlorobenzilate	On screen
Chlorothalonil	On screen
Dicofol	On screen
Ethalfluralin	On screen
Folpet	On screen
Lindane	On screen
Methomyl	On screen
Oxadiazon	On screen
Parathion	On screen
PCNB	On screen
Pronamide	On screen
Tetrachlorvinphos	On screen
Toxaphene	On screen
Trifluralin	On screen

TABLE 2-10. California Department of Food and Agriculture Activity on Pesticides Considered by EPA as Animal Carcinogens (Continued)

Pesticide	Activity
Acephate	Through screen[a]
Amitraz	Through screen[a]
Azinphos-methyl	Through screen[a]
Captafol	Through screen[a]
Captan	Through screen[a]
Cypermethrin	Through screen[a]
Diclofop methyl	Through screen[a]
Linuron	Through screen[a]
Permethrin	Through screen[a]
Benomyl	Focused monitoring (currently)
Mancozeb	Focused monitoring (currently)
Maneb	Focused monitoring (currently)
Zineb	Focused monitoring (currently)
Metiram	Focused monitoring (1988-89)
o-Phenylphenol	Focused monitoring (1988-89)
Maleic hydrazide	Focused monitoring (1988-89)
Thiophanate methyl	Focused monitoring (1988-89)
Paraquat	Focused monitoring (1988-89)
Metolachlor	Focused monitoring (1988-89)

TABLE 2-10. California Department of Food and Agriculture Activity on Pesticides Considered by EPA as Animal Carcinogens (Continued)

Pesticide	Activity
Calcium arsenate	No Calif. registration. EPA cancellation proposed.
Chlordimeform	No Calif. registration. EPA cancellation proposed.
Copper arsenate	No Calif. or Federal registration. EPA cancellation proposed.
Cyromazine	No Calif. registration.
Diallate	No Calif. registration.
Lead arsenate	No Calif. or Federal registration. EPA cancellation proposed.
Sodium arsenate	No Calif. registration. Federal registration for non-crop uses only.
Fosetyl al	Low sales (technical material only).
Thiodicarb	No reported sales in California.
Terbutryn	Low sales. Use as pre-emergent herbicide.
Acifluorfen	No use on fresh market foods.
Arsenic acid	No use on fresh market foods.
Asulam	No use on fresh market foods.
Ethylene oxide	No use on fresh market foods.
Glyphosate	No direct application to crop (herbicide).
Methanearsonic acid	No direct application to crop (herbicide).
Oryzalin	No direct application to crop (herbicide).
Daminozide	U.S. Food and Drug Administration monitoring.
Sodium arsenite	Dormant usage only.

[a] Qualitatively identified on screen, follow-up quantification by specific (single residue) analysis.

  - Residues less than 50.0% of tolerance were detected in 17.7% of the samples.

  - Residues between 50.0% and 100.0% of tolerance were detected in only 1.0% of the samples.

  - 1.5% of the samples were out of compliance.

- Most samples not in compliance (1.2%) contained pesticides for which no tolerance was established. Eighteen of the samples taken (0.3%) were found to have residues in excess of the established tolerance.

- Two hundred different commodities were sampled. These included major production commodities (tomatoes and grapes), and unusual crops, such as edible flowers.

- When illegal residues are detected, commodities are immediately quarantined and not allowed to be sold. The field, if in California, is sampled and also quarantined if illegal residues are detected.

- Sampling of foreign produce increased. In 1988, over 29% of the samples were from foreign countries. Mexican (1,665 samples) and Chilean (202 samples) commodities were most frequently sampled.

- CDFA instituted a new program in 1987 to target commodities at the points of entry into California. In 1988, samples were taken for the first time at the border stations between California and Mexico, and from ship docks and airports in Los Angeles.

Focused Monitoring Including Unique Subpopulation Dietary Profiles

- The focused monitoring program targets pesticides for which concerns of possible adverse health effects have been identified. Commodities for sampling are selected after careful consideration of pesticide usage, volume of production, and the diets of various population subgroups. Only fields which have been treated with the targeted pesticides are sampled.

- During 1987, 22 pesticides were targeted in this program, with analyses completed on over 970 samples.

- Ninety per cent of the samples contained no detectable residues. The remaining 10.0% contained levels far below the established tolerances. No samples contained residues over the allowable limit.

During 1987, 1,146 samples were taken of produce destined for processing, resulting in 114,600 analyses of 50 different commodities. Of these samples, 82.8% contained no detectable residues. The compliance rate in this program was 99.7% for 1987.

During 1987, 4,317 preharvest samples were taken, resulting in approximately 431,700 analyses of over 160 different commodities. Only 0.5% of these samples contained residues of pesticides which could not be legally used on the commodity. None of the commodities sampled in this program contained over-tolerance residue levels at harvest. Thirty-five of the chemicals were included in the Department's Integrated Pesticide Residue Program for FY 87/89. Over 168,000 analyses for these chemicals were performed on over 7,000 samples in 1987. Only 0.2% of the samples contained detectable levels of the pesticides, and only 0.001% of the samples were found to contain levels over the established tolerance.

All nations have subpopulations with unique dietary profiles, and many nations also have relatively large groups of unique ethnic populations or cultures dependent on radically different geographical limitations on their dietary supplies (e.g., mountain communities versus coastal regions). Socioeconomic differences within a nation will also heavily influence dietary patterns. The same data mentioned above usually include information which allows creation of subgroup exposure profiles.

The dietary habits of any nation may be unique to that nation for many reasons. Ethnic heritage may favor certain commodities and may even prohibit consumption of other commodities. Geographical factors make some foods readily available while rendering others available only via importation--usually associated with lower consumption due to limited availability and increased cost. The form in which these foods is consumed may vary greatly among countries. The extent of cooking, form of preparation and extent of commercial preparation vary greatly. Since preparation may affect the nature and concentration of the chemicals in foods, such variation influences the overall exposure

The extent of exposure via secondary residues in animal products from animals fed by-products of local crop commodities in their feed (e.g., apple pomace remaining after juice extraction) is also influenced by the local agricultural practices. Secondary residues may be a major concern in some areas while contributing little or nothing to the overall dietary exposure in other countries where dairy, poultry or meat production is less dependent on the use of agricultural by-products.

Economic factors that dictate the extent and cost of imported food and the source of such imports alter consumption profiles and the nature of the chemicals in those foods. Storage time, shipment methods, geographical source and pesticide use at those locations, degree of processing of imports and cost of imports in relation to cost of domestic substitutes influence the exposure profile. National nutritional aid programs also alter the dietary profiles of some national groups. Food subsidy and food supplement programs influence the dietary choices of the populations targeted for such programs.

For all of these variables, economists, nutritionists, business bureaus and commerce agencies have excellent records, which are available to health professionals who must define the national dietary profiles and estimate the pesticide content in those consumed foods. These records preclude the need for making gross assumptions about regional or national dietary habits. For almost every country or geographical region, national food consumption surveys, nutritional surveys or food sale records are maintained. They may reside in any of a variety of institutions--federal agencies, academic institutions, commerce alliances, international aid agencies, consumer advisory groups, food processors and food retailers, or other sources. It is relatively easy to construct detailed quantitative descriptions of population consumption profiles.

Household Exposure

The principal exposure of the general U.S. population to pesticides occurs in the home. Studies have shown that about 90% of all U.S. households use pesticides (EPA, 1979; Kiel et al., 1969; Savage et al., 1981; von Rumker et al., 1974). A national survey conducted by the U.S. Environmental Protection Agency during 1976-77 (EPA, 1979) revealed that of the 90% of the U.S. households that used pesticides, 83.7% used them inside the house, 21.4% in the garden, and 28.7% on the lawn. Twelve of the 14 most commonly used pesticides were insecticides. Over 90% of the households used disinfectants (antimicrobials), 36% used moth repellents, and 26% were treated with termiticides. The annual use of insecticides in U.S. homes has been roughly estimated at 28.5 million kg, and antimicrobials at 126.6 million kg (Reinert, 1984). However, these estimates may be low based on 1980 estimates by the State of California that 20.6 million kg of pesticides were sold for use in the home (including termiticides) and 8.8 million kg for home garden use (CDFA, 1982). The figures represent 16% of the total pesticide usage in that state (174 million kg). They exclude antimicrobials used for treatment of domestic water supplies, sewer treatment systems, and swimming pools (69 million kg). Total pesticide sales in California increased by 12.7% from 1980 to 1986 (CDFA, 1988).

A wide variety of pesticide products are available "off the shelf" for use by the homemaker. These include preparations for flies, roaches, ants, and spiders within the home, flea and tick sprays, powders, collars, and shampoos for pets, insecticides for use on house plants and home gardens, insecticides and fungicides for lawn treatment, and a variety of herbicides for weed and crabgrass control. While most homemakers do not consider disinfectants to be pesticide products, most use them routinely as kitchen and bathroom cleaners, room deodorizers, or laundry aids. Many homeowners and landlords utilize professional pest control services for routine indoor treatments or lawn care. In many parts of the country, especially in the southern states, pre- or post-construction treatment for termite protection is essential.

During 1976-1977 (EPA, 1979), it is estimated that exposure to pesticides around the home resulted in 2.5 million reported incidences of headache, nausea, dizziness, and eye or skin irritations. Exposure to pesticides in the home accounted for 75% of all pesticide-related injuries treated in hospital emergency rooms in 1981 (Reinert, 1984). In California during 1983-1986, nearly 300 cases of illness or injury reported to physicians were attributed to three popular household insecticides: chlorpyrifos, dichlorvos (DDVP), and propoxur (Edmiston, 1987). Of greater concern, however, are potential chronic health effects that may derive from long-term residential exposures.

A typical U.S. resident spends the large majority of time indoors, mostly inside his or her own domicile (Figure 2-1). Full-time homemakers and small children, may spend over 21 hours/day inside the home (Robinson, 1977), with another 2.5 hours inside other buildings (stores, offices, etc.) or in transit vehicles. Even when employed, urban dwellers spend about the same amounts of time indoors, 63% of it at home and 28% at work. For many persons, at least 90% of the daily cumulative exposure to pesticides occurs at home.

Some pesticides persist in the home environment for many months or years after application. This is true of termiticides, whose efficacy depends on their persistence. Chlordane has been found to be highly effective for over 20 years after treatment. Even non-persistent pesticides last much longer indoors where they are protected from environmental factors that hasten their degradation.

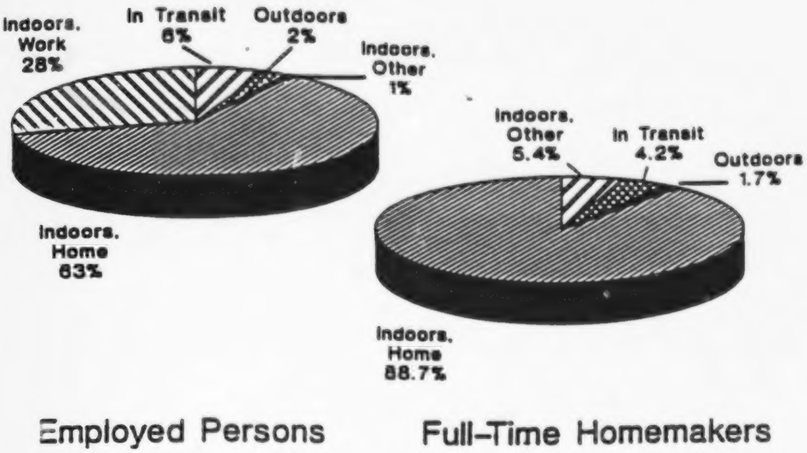

FIGURE 2-1. Proportion of time spent in various locations.

Residues of these pesticides found on floors and other surfaces and in the air inside the home contribute significantly to the overall exposure of the general population. Results from personal exposure monitoring of adults in Jacksonville, Florida showed that 85% of the total daily exposure to airborne pesticides was from breathing air inside the home (Lewis et al., 1986, 1988). Dislodgeable pesticide residues in carpets or on uncovered floors appear to be a major route of exposure for infants and toddlers through dermal and oral ingestion.

Methodology for measurement of most pesticides expected to be found in air is generally good and a good database exists for indoor air levels of many pesticides found. On the other hand, information needed for assessment of the relative importance of oral, dermal, and inhalation routes of exposure to household pesticides is fragmentary. Monitoring methods for dermal exposure are inadequate, and only recently has a satisfactory house dust sampler been developed.

Contributions from dietary and drinking water intake have not generally been studied in the same context with other routes of exposure. The U.S. EPA has undertaken an investigation of exposure to household pesticides under its long-range total human exposure assessment program. This study, known as the Non-occupational Pesticide Exposure Study (NOPES), was designed to evaluate methodology and to provide some indication of the relative importance of the different exposure routes.

Indoor Air. Of the several possible routes of exposure to household pesticides, the air route may be the most significant. For example, the use of a pesticide spray product indoors has been estimated to result in an average air concentration of 0.7 $\mu g/m^3$ of the active ingredient in air over a three-day period following application (Reinert, 1984). Residents spending 15 to 20 hours/day inside would then receive total exposures to the pesticide of 19 to 25 μg per application (assuming an average breathing rate of 10 liters/minute).

In addition to the periodic introduction of pesticides into household air by direct application, there are often sources that continually emit vapors into the living space. These include intrusion of termiticides from the foundations of the dwelling (Leidy et al., 1985), evaporation of residues from crack and crevice treatments (Wright and Jackson, 1976), emissions from impregnated pest control strips often used inside kitchen cabinets (Jackson and Lewis, 1981; Leary et al., 1974), vapors from moth repellents and room deodorizers (Wallace, 1987), and contamination from house dusts and soils tracked into the home (Lewis and Lee, 1976).

At any given time, the air inside of the average dwelling in the U.S. contains several kinds of pesticides at levels that are typically 10 to 100 times higher than those found in the immediately surrounding outdoor air (Lewis and Lee, 1976; Lewis and MacLeod, 1982; Lewis et al., 1986). A 1985 study by EPA in Jacksonville, Florida, showed that 81% of the total respiratory exposures to 24 of 33 commonly used household pesticides occurred inside the home (Lewis et al., 1986, 1988).

Concern over the potential chronic health effects from exposure to termiticides in indoor air has prompted the National Research Council (NRC, 1982a) and the Environmental Protection Agency (EPA, 1983) to issue risk assessment documents. In the NRC document, interim guidelines were set for airborne exposure to five termiticides: chlordane (5 $\mu g/m^3$), heptachlor (2 $\mu g/m^3$), aldrin (1 $\mu g/m^3$), dieldrin (1 $\mu g/m^3$), and chlorpyrifos (10 $\mu g/m^3$). Maximum indoor air concentrations set by the NRC are shown in Table 2-11.

The first major study of termiticides in residential indoor air was conducted by the U.S. Air Force (Lillie, 1981). Of 435 treated apartments at one Air Force facility, 77% showed measurable air levels of chlordane. Mean concentrations were in the 1 to 10 $\mu g/m^3$ range for apartments treated 10 to

TABLE 2-11. Insecticides Found in Indoor Air in U.S. Households

Compound	NRC Guideline[a] ($\mu g/m^3$)	Measured Concentration ($\mu g/m^3$) Range	Mean[b]	Location[c]	Reference
Chlordane	5	>0.2 - 37.8	--	Throughout U.S.	Lillie (1981)
		0 - 264	--	Throughout U.S.	Livingston & Jones (1981)
		0.3 - 5.8	1-10	N.C.	Wright & Leidy (1982)
		0.2 - 5.5	--	N.C.-S.C.	Lewis & MacLeod (1982)
		0 - 2.3	--	Cal.	EPA (1983)
		0 - 3.6	--	Colo.	EPA (1983)
		0.05 - 9.9	3	N.C.	Leidy et al. (1985)
		0 - 130	--	Throughout U.S.	Olds (1987)
		0.2 - 5.6	1.6	Fla.	Lewis et al. (1987)
		0.01 - 52	--	W. Va.	Qazi (1987)
		0.7 - 9.9	--	N.C.	Qazi (1987)
		0.01 - 3.7	--	Colo.	Qazi (1987)
		0 - >5	--	Throughout U.S.	Lillie & Barnes (1987)
		0 - 1.7	0.6	Fla.	Lewis et al. (1988)
		0 - 4.4	0.3	Fla.	Immerman and Firestone (1989)
		0 - 1.7	0.2	Mass.	Immerman and Firestone (1989)

TABLE 2-11. Insecticides Found in Indoor Air in U.S. Households (Continued)

Compound	NRC Guideline[a] (μg/m³)	Measured Concentration (μg/m³)		Location[c]	Reference
		Range	Mean[b]		
Heptachlor	2	0.01 - 1.8	--	N.C.	Wright & Leidy (1982)
		0	- 0.6	--	Colo.EPA (1983)
		<0.1	- 14.8	--	Va.Jurinski (1984)
		0.02 - 2.0	1.6	N.C.	Leidy et al. (1985)
		0.02 - 1.6	0.4	Fla.	Lewis et al. (1987)
		0 - 0.4	0.18	Fla.	Lewis et al. (1988)
		0 - 2.4	0.14	Fla.	Immerman and Firestone (1989)
		0 - 0.2	0.03	Mass.	Immerman and Firestone (1989)
Aldrin	1	<0.04 -	7.0	--	--EPA (1983)
		0 - 1.55	--	Throughout U.S.	Olds (1987)
		0.1 - 5	--	Throughout U.S.	Jaquith et al. (1987)
		0 - 0.14	0.01	Fla.	Lev-On et al. (1987)
Dieldrin	1	<0.04 - 0.17	--	--	EPA (1983)
		0 - 0.11	--	Colo.	EPA (1983)
		0 - 0.02	0.04	Fla.	Lev-On et al. (1987)

TABLE 2-11. Insecticides Found in Indoor Air in U.S. Households (Continued)

Compound	NRC Guideline[a] ($\mu g/m^3$)	Measured Concentration ($\mu g/m^3$) Range	Mean[b]	Location[c]	Reference
Diazinon	--	0.4 - 38	--	N.C.	Leidy et al. (1982)
		0.01 - 2.0	--	N.C.-S.C.	Lewis & MacLeod (1982)
		<0.1 - 149	--	N.C.	Leidy et al. (1984)
		0 - 34.6	--	Throughout U.S.	Olds (1987)
		0.06 - 13.7	1.0	Fla.	Lewis & Bond (1987)
		0.03 - 8.8	1.4	Fla.	Lewis et al. (1988)
		0 - 3.0	0.26		
		0 - 1.8	0.05		
Propoxur	--	0.11 - 0.79	--	N.C.	Jackson & Lewis (1981)
		0.02 - 7.9	0.4	Fla.	Immerman and Firestone (1989)
		0 - 2.0	0.03	Mass.	Immerman and Firestone (1989)
		0.02 - 0.7	0.16	Fla.	Lewis et al. (1988)
Dichlorvos	--	0.05 - 28	--	N.C.-S.C.	Lewis & MacLeod (1982)
		0 - 2.9	0.12	Fla.	Immerman and Firestone (1989)
		0 - 0.3	0.0003	Mass.	Immerman and Firestone (1989)

TABLE 2-11. Insecticides Found in Indoor Air in U.S. Households (Continued)

Compound	NRC Guideline[a] ($\mu g/m^3$)	Measured Concentration ($\mu g/m^3$)		Location[c]	Reference
		Range	Mean[b]		
Naphthalene	--	0.55 - 4.2	--	Ohio	Wilson & Chuang (1987)
p-Dichlorobenzene	--	0 - 1500	2.3	Throughout U.S.	Wallace (1987)
Pentachlorophenol	--	0.1 - 0.5	--	N.C. (log homes)	Lewis & MacLeod (1982)
		0 - 0.3	0.03	Fla.	Immerman and Firestone (1989)
		0.9 - 50	--	Cal. (offices)	Levin & Hahn (1984)
Chlorpyrifos	10	0.01 - 7.0	--	N.C.-S.C.	Lewis & MacLeod (1982)
		2 - 37	--	Throughout U.S.	EPA (1983)
		0.01 - 15	2.4	Fla.	Lewis et al. (1986, 1988)
		0.02 - 2.2	0.5	Fla.	Lewis & Bond (1987)
		0.7 - 8.5	1.1	N.C.	Leidy & Wright (1987)
		0.04 - 4.5	--	Fla.	Bush et al. (1987)
		0 - 4.4	0.3	Fla.	Immerman and Firestone (1989)
		0 - 1.7	0.0007	Mass.	Immerman and Firestone (1989)

TABLE 2-11. Insecticides Found in Indoor Air in U.S. Households (Continued)

Compound	NRC Guideline[a] ($\mu g/m^3$)	Measured Concentration ($\mu g/m^3$)		Location[c]	Reference
		Range	Mean[b]		
Malathion	--	0.1 - 0.0	--	N.C.-S.C.	Lewis & MacLeod (1982)
		0 - 13.7	0.02	Fla.	Immerman and Firestone (1989)
		0 - 1.8	0.003	Mass.	Immerman and Firestone (1989)
Carbaryl	--	0 - 0.1	0.07	Fla.	Immerman and Firestone (1989)

[a] Values listed for the five termiticides covered by NRC guidelines.

[b] Dash indicates no mean data published.

[c] Dash means that location of sampling not indicated.

15 years prior to sampling (Livingston and Jones, 1981). In a study of 4,776 Air Force housing units, 5% were found to exceed the NRC guideline (EPA, 1983). Large-scale surveys by the Army and Navy Departments showed that 0.9 and 1.4% of their housing units, respectively, exceeded the guideline (EPA, 1983; Olds, 1987). Air monitoring studies in non-military housing has likewiseshown chlordane and heptachlor at low $\mu g/m^3$ levels, with occasional measurements above the NRC guidelines.

Aldrin and dieldrin are equal to chlordane in persistence and are five times as mutagenic (NRC, 1982a). Their use has been more limited and monitoring studies have generally shown the indoor level of these termiticides to be lower than those found for chlordane.

Chlorpyrifos is currently found more frequently in the average U.S. household than other pesticides that are used as termiticides (Bond and Lewis,1987; Lewis and Bond, 1987). However, this insecticide is much more widely used as a household insecticide (both for professional treatments and by homeowners) than as a termiticide. While it may soon become the only subterranean termiticide approved in the U.S., exposures resulting from its use as a general purpose insecticide will likely remain the more important concern in the foreseeable future.

Since termiticides are applied to the soil around and under the foundation of the house, penetration through the floors and walls is the major route of intrusion. Evidence suggests that crawl space homes exhibit higher indoor air concentrations than slab homes. Chlordane air levels exceeded 5 $\mu g/m^3$ in 19% of Air Force dwellings built over crawl spaces compared to 5% for slab houses with in-slab ducts and 0% for slab houses with attic ducts (Lillie and Barnes, 1987). Mean and maximum chlordane levels were five times higher for crawl space houses than slab homes in studies conducted by Velsicol Chemical Corporation in 1979 (EPA, 1983). In a similar study conducted by Shell Research Limited (EPA, 1983), aldrin levels were four to five times higher for crawl space houses. Plenum houses, which use the crawl space as part of the air distribution system exhibited higher termiticide concentrations than standard crawl space houses or slab houses (Jaquith et al., 1987).

Air concentrations of termiticides in basements generally exceed those found in upper-floor rooms. Jaquith et al. (1987) reported aldrin levels that were 10 times higher in the basement than in first-floor rooms one day after treatment. The difference had dropped to a factor of about five after 90 days. When chlorpyrifos was used for subterranean termite control, air levels in the living area of basement houses were six to ten times as high as in crawl space houses. Jurinski (1984) measured airborne heptachlor and chlordane in basement, first-, and second-floor rooms, and found basement levels typically higher. In one case, he calculated that a child spending 1.3 hours in the basement would receive an exposure to heptachlor of 0.5 μg/kg body weight/day.

Since chemicals used for subterranean termite control and radon enter the living spaces of dwellings by the same route, effective mitigation techniques should be the same or very similar.

Common household pesticides detected in indoor air in the United States are shown in Table 2-11. Based on manufacturing and sales estimates, the major pesticides used in U.S. homes are naphthalene, pentachlorophenol and *p*-dichlorobenzene. The former and latter are used as moth repellents and the latter two are used primarily as antimicrobials. Few data exist on naphthalene levels in indoor air. *p*-Dichlorobenzene has been shown to be ubiquitous in indoor air (Wallace, 1987).

Pentachlorophenol (PCP) is also a widespread environmental pollutant. It is estimated that PCP and its conjugates are detectable in the urine of about 72% of the general population of the United States (personal communication from O. Carter, National Center for Health Statistics, Hyattsville, Md., 1988). While it is obvious that exposure to PCP in the United States is extensive, there is little or no information on the extent of exposure in and around the household. Significant air levels have been reported inside office buildings (Levin and Hahn, 1984). PCP is used as a wood preservative, principally on porches and patio decking. It is also used as a fungicide to protect leather goods, some paints, and other materials which may be used inside the home. In Europe, where use of PCP in indoor paints and stains has been common, indoor exposure via the air route is considered potentially serious (e.g., Maroni et al., 1987). Except for incidences relating to exposures of residents of log homes (Strassman et al., 1985), there have been few investigations of home air quality in the United States.

Insecticides in common use around the household are: chlorpyrifos, diazinon, propoxur, dichlorvos, malathion, carbaryl, pyrethrins (natural and synthetic), and piperonyl butoxide. Chlorpyrifos is currently the most widely used as a broad-spectrum insecticide and is being used with increasing frequency for subterranean termite control. It has been found in the air inside most of the households monitored for it. In several incidences, the NRC guideline of 10 μg/m^3 has been exceeded (see Table 2-11). Diazinon, propoxur, and malathion follow closely behind chlorpyrifos in frequency of detection.

Dichlorvos (DDVP) has been used as a household insecticide for many years. Slow-release insecticide strips containing this chemical were popular in the United States in the 1970's. Several studies carried out during that time showed that air concentrations greater than 100 $\mu g/m^3$ were not unusual in rooms in which these strips were deployed (Leary et al., 1974; Lewis and Lee, 1976). Meals and food items also became contaminated via air transport in these rooms (Lewis and Lee, 1976). Using a "worst-case" scenario, the EPA has calculated that lifetime exposures to dichlorvos vapors from peststrips may result in a 10^{-2} cancer risk (Consumer Reports, 1988). The total daily average exposure to a 70-kg resident spending 15 hours per day in the home and breathing 12.9 m^3 was estimated to be 10 $\mu g/kg$ body weight (Jaquith, 1987). Gold and Holsclaw (1985) estimated from room air measurements and blood acetylcholinesterase determinations an average exposure of 0.03% of the toxic dose/hour for occupants of homes during and for a full 24 hours after professional application of dichlorvos.

Carbaryl has been detected in indoor air at low concentrations. Permethrin and resmethrin have been rarely found and only at very low levels (0.001 $\mu g/m^3$ or less). No indoor monitoring data on piperonyl butoxide were found in the literature. A few homeowners use lindane for pine bark beetle control and for other insects in the yard. Lindane and other insecticides (e.g., chlorpyrifos) may also be found in firewood. Residential wood combustion may introduce these insecticides inside the home (Bush et al., 1987).

The most commonly used household disinfectants that may contribute to indoor air pollution are pine oil and phenols. α-Pinene, a component of pine oil, has been recognized as a ubiquitous indoor air constituent for several years. Average concentrations in 539 U.S. households have been reported to be 2.4 $\mu g/m^3$ (Wallace, 1987). *Ortho*-Phenylphenol (the active ingredient in some Lysol^R disinfectants) and 2-benzyl-4-chlorophenol (BCP) are the most popular phenolic antimicrobials. Reinert (1984) estimated an annual use of 680,000 kg of *o*-phenylphenol and 5.3 million kg of BCP in U.S. homes. In California, total sales in 1986 amounted to 146,000 kg of *o*-phenylphenol as the free acid and 102,576 kg as the sodium and potassium salts (CDFA, 1988). By comparison, 88,563 kg of free BCP was sold, along with only 5,331 kg of the salts. A review of the literature revealed little information on indoor air levels of phenolic disinfectants. Lewis et al. (1986) reported finding *o*-phenylphenol in five of nine Jacksonville, Florida, homes during August 1985. A survey of pesticide products also revealed cans of Lysol^R disinfectant spray in five of these households; however, detection of *o*-phenylphenol in the air did not always coincide with finding Lysol^R spray on the premises. Data from a more comprehensive report of the same EPA study are given in Table 2-12a and Table 2-12b (Immerman and Firestone, 1989).

TABLE 2-12a. Major Pesticides Found in Indoor Air in the EPA Non-Occupational Pesticide Exposure Study: Jacksonville, Florida

Pesticide	Summer 1986			Spring 1987			Winter 1988		
	% of Households	Concentration µg/m³		% of Households	Concentration µg/m³		% of Households	Concentration µg/m³	
		Mean	Max.		Mean	Max.		Mean	Max.
Chlorpyrifos	100	0.37	2.2	88	0.20	4.4	96	0.12	1.04
Propoxur	98	0.53	7.9	93	0.22	2.0	95	0.16	1.37
o-Phenylphenol	85	0.10	1.0	84	0.07	1.2	79	0.06	1.44
Diazinon	83	0.42	3.0	83	0.11	2.4	83	0.08	0.52
Chlordane	62	0.32	3.0	54	0.25	4.4	94	0.22	1.71
Heptachlor	58	0.16	1.6	71	0.15	2.4	92	0.07	0.68
Dichlorvos	33	0.13	2.3	14	0.09	2.9	10	0.02	1.09

TABLE 2-12b. Major Pesticides Found in Indoor Air in the EPA Non-Occupational Pesticide Exposure Study: Springfield, Massachusetts

Pesticide	Summer 1987			Winter 1988		
	% of Households	Concentration $\mu g/m^3$		% of Households	Concentration $\mu g/m^3$	
		Mean	Max.		Mean	Max.
Chlorpyrifos	29	0.01	0.25	31	<0.01	0.29
Propoxur	49	0.03	0.50	38	0.02	0.67
o-Phenylphenol	91	0.04	0.56	72	0.03	0.29
Diazinon	16	0.05	1.80	10	<0.01	0.03
Chlordane	50	0.20	1.70	83	0.03	0.74
Heptachlor	14	0.03	0.25	70	<0.01	0.15
Dichlorvos	2	<0.01	0.32	1	<0.01	0.16

Other Household Sources of Pesticide Exposure Herbicides are unlikely to be used inside the home. However, several are commonly used for lawn care and may enter the home via intrusion of outdoor air or may be carried in on soil particles. Phenoxycarboxylic acid herbicides, glyphosate and dicamba are most commonly used. 2,4-Dichlorophenoxyacetic acid (2,4-D) and 2(2-methyl-4-chlorophenoxy)propionic acid are the most popular of these. Calcium acid methanearsonate (CAMA), dimethylarsenic acid, cacodylic acid (sodium salt), pramitol, diquat and triclopyr are used for edging, crabgrass control, poison ivy elimination and other special purposes by homeowners. None of these compounds have an appreciable vapor pressure and would not be expected to present an inhalation exposure hazard except during spray applications. Two 2,4-D esters that possess sufficient vapor pressures to be found in air were sought in a NOPES study. In only a few incidences have the butoxyethyl and methyl esters been detected in indoor or outdoor air, always at very low (0.001 $\mu g/m^3$ or less) levels (Immerman and Firestone, 1989).

Rodenticides are also frequently used in and around the home, generally added to baits. The potential for air route exposure is essentially non-existent. Accidental ingestion, especially by small children, is the most serious potential health risk associated with rodenticides.

Insect repellents, such as Deet (N,N-diethyl-*m*-toluamide, the active ingredient in OFF^R), may also be vaporized into air, especially when applied as sprays. However, dermal exposure to these compounds is of primary concern.

Approximately 1200 inert ingredients are currently registered for use in pesticide formulations. These include solvents propellants, emulsifiers, and adjuvants. Toxicological data are available for only 400 of these additives. EPA has serious concerns about potential health risks that may be associated with about 120 of these, and has issued a policy statement requiring labelling and possible cancellation (EPA, 1987).

Two lists of inert ingredients were in the EPA announcement. One listed 57 chemicals of toxicological concern because of known carcinogenicity, adverse reproductive effects, neurotoxicity or other chronic effects, or developmental toxicity. These include chlorinated solvents, (e.g., methylene chloride, chloroform and carbon tetrachloride), benzene, *n*-hexane, dioxane, cellusolve, and epichlorhydrin. The other listed a similar number of potentially toxic compounds that have been given high priority for testing under the National Toxicology Program. Included in the second list are chlorofluorocarbons, 1,1,1-trichloroethane (methylchloroform), toluene, xylenes, acetonitrile, chloroanilines and phthalates. Such ingredients are commonly found in household pesticide formulations in amounts 50 to 100 or more times greater than the active (pesticidal) ingredient. The potential health risks from exposure to the inert ingredients may often outweigh those associated with pesticides themselves.

EPA and several other agencies have established an interagency workgroup on chlorinated solvents. This group is currently assessing the potential risks associated with household exposures to such solvents used in pesticidal and sanitizer aerosol products (D. A. Jaquith, EPA Office of Pesticides and Toxic Substances, personal communication, 1988).

Seasonal and Geographic Considerations Most household pesticides are used during spring and summer months when pest infestations are greatest. Consequently, air concentrations should be highest during these times. Warmer weather may also increase volatilization of termiticides from the soil beneath the house resulting in increased contributions to living spaces. Since insect populations are generally larger in more moderate climates, pesticide usage and corresponding exposure levels should be greater in the southern states.

In its Non-Occupational Pesticides Exposure Study (NOPES), EPA is conducting the first large-scale study of household pesticides exposure across the United States (Lewis and Bond, 1987). This Congressionally mandated study, which was initiated in 1985, was carried out in two suburban-urban population centers selected to represent high and low household pesticide usage. Cities and states were screened on the basis of usage data obtained from the National Urban Pesticides Applicator Survey conducted during 1983-84 (Immerman and Drummond, 1984). Jacksonville, Florida, was chosen as the high-use city and Springfield, Massachusetts, was selected as the representative low-use city. Both are considered to be in non-agricultural regions. At each location, households were stratified prior to monitoring into low-, medium-, and high-use categories. Approximately 175 homes in Jacksonville and 85 homes in Springfield (representing approximately 300,000 and 140,000 residents, respectively) were chosen for study of air, dermal, drinking water, and dietary exposures to 33 of the most commonly used household pesticides. Exposure measurements were made during the spring, summer, and winter seasons.

In Jacksonville, where three seasons of monitoring were conducted, 25 to 27 of the targeted pesticides were detected in indoor air. Total mean air concentrations were 2.3 $\mu g/m^3$ in summer, 1.2 $\mu g/m^3$ in spring, and 0.81 $\mu g/m^3$ in winter. Outdoors (on porches and patios), 9 and 16 at total mean concentrations of 0.11, 0.03 and 0.06 $\mu g/m^3$ for summer, spring and winter, respectively. Summertime monitoring was not performed in Springfield. During spring and winter, 20 and 16 pesticides were found indoors at total mean air concentrations of 0.40 and 0.10 $\mu g/m^3$ respectively. Ten and six were found outdoors at 0.03 and 0.01 $\mu g/m^3$, respectively. The seven most prevalent pesticides are shown in Table 2-12a and Table 2-12b. Springtime indoor air levels were higher by a factor of 3 in Jacksonville, while wintertime concentrations were 8 times higher.

The average homemaker may receive dermal exposure to pesticides when spraying insecticides inside the home, shampooing their pets with flea and tick preparations, mixing emulsifiable concentrates for ornamental, lawn, or garden applications, and the application of insecticides, herbicides, and fungicides in the yard. Intentional dermal applications may occur. For example, treatment for head lice may involve shampoos such as Kwell, which contains lindane.

Chronic respiratory exposure probably results in greater annual cumulative doses than dermal contact for most households. Frequent bathing of animals with flea and tick shampoos without proper hand protection may be an exception.

House dust and the pollutants carried with it may be potentially important contributors to exposure through inhalation, ingestion, and skin penetration, especially for small children. Dust can be both a medium for the transfer of pesticides from sources inside and outside the house to people and a medium for their accumulation. Starr et al. (1974) first tested house dust for pesticides in 28 homes of farmers and pesticide formulators in Colorado. He found that the concentrations of organochlorine pesticides were typically in the 10 to 100 ppm range. Klemmer et al. (1975) found arsenic in house dust from 61 homes in Hawaii that had been treated with arsenical pesticides. Arsenic levels ranged from 1 to 1100 ppm. Davies et al. (1975) studied exposure to pesticides in house dust by 254 residents of the Caribbean Island of Bimini. They found that the average pesticide levels in house dust were 15 to 50 times higher than those in the soils immediately surrounding the dwellings. Air levels of *p,p'*-DDT were found to be elevated for 30 minutes after sweeping. Exposure to DDT in house dust was estimated to exceed that received from the diet. While not solely associated with house dust, chlordane residues ranging from 0.08 to 2.2 mg/m^2 have been reported on floors and carpets in treated homes (Jurinski, 1984; Wright and Leidy, 1982). Dichlorvos and propoxur residue as high as 68 mg/m^2 and 155 mg/m^2, respectively, have been found on floors in rooms treated with an indoor fogger (Maddy et al., 1987).

Toddlers (under age 5) have only about 20% of the body weight of an adult, yet ingest 2.5 times as much dust (Hawley, 1985). Therefore, the potential health risk to children is about 12 times that to adults. Some research has suggested that dust ingestion may account for 1000 times more exposure than that from inhalation to long-lived soil contaminants by young children (Roberts et al., 1985). For stored pollutants, childhood intake may represent a major source of the total adult body burden. There have been several studies that have shown house dust to have significant mutagenicity (Roberts et al., 1987a). However, it has been impossible to accurately estimate the risks associated with these exposures because house dust sampling methodology has been inadequate, especially for organics, and insufficient data have been obtained on simultaneous air and dermal exposures, the latter of which is also very difficult to measure.

EPA has recently sponsored the development of a sampler designed to collect surface dust in a controlled manner and maintain the integrity of the pesticides associated with it (Roberts et al., 1987b; Ruby et al., 1987). The sampler can collect 2 to 4 grams of floor dust from a typical dwelling floor (generally carpeted) in about 10 minutes. The device was used to collect samples in Jacksonville, Florida in February 1988 as part of the winter monitoring for the NOPES program. Analysis of the collected dust showed high concentrations (1 to 100 ppm) of many pesticides. In older carpets, long-banned pesticides such as DDT were found at ppm levels (Budd et al., 1989).

If the estimate by Binder et al. (1986) for daily soil ingestion by small children of 0.12 to 1.8 g is applied to house dust, some crude estimates of relative exposure via the oral ingestion and inhalation routes may be made. Assuming a pesticide level on house dust of 10 ppm, an average air concentration of 0.5 $\mu g/m^3$, and a respiration rate of 3 liters/min, then a child ingesting one gram of house dust per day would take in 10 μg/day orally and 2 μg/day by respiration. It appears, therefore, that house dust may represent a very important route of exposure of small children to pesticides.

Drinking Water In 1986, EPA reported the results of groundwater monitoring for 17 different pesticides in 23 states (EPA, 1986b). These findings are presented in Table 2-13. Water samples were collected from each of the water sources used by the participants of the EPA NOPES program in Jacksonville, Florida and Springfield, Massachusetts. During the spring 1987 phase of the study, the only pesticide detected in Jacksonville was 0.06 μg/liter of diazinon in one private company sample. No pesticides were detected during the summer season.

TABLE 2-13. Typical Positive Results of Pesticide Ground-Water Monitoring in the U.S. as a Result of Normal Land Application[a]

Pesticide	State(s)	Pesticide Concentration (μg/L)
Alachlor	MD, IA, NE, PA	0.1-10
Aldicarb (sulfoxide and sulfone)	AR, AZ, CA, FL, MA, ME, NC, NJ, NY, OR, RI, TX, VA, WA, WI	1-50
Atrazine	PA, IA, NE, WI, MD	0.3-3
Bromacil	FL	300
Carbofuran	NY, WI, MD	1-50
Cyanazine	IA, PA	0.1-1.0
DBCP	AZ, CA, HI, MD, SC	0.02-20
DCPA (and acid products)	NY	50-700
1,2-Dichloropropane	CA, MD, NY, WA	1-50
Dinoseb	NY	1-5
Dyfonate	IA	0.1
EDB	CA, FL, GA, SC, WA, AZ, MA, CT	0.05-20
Metolachlor	IA, PA	0.1-0.4
Metribuzin	IA	1.0-4.3
Oxamyl	NY, RI	5-65
Simazine	CA, PA, MD	0.2-3.0
1,2,3-Trichloropropane	CA, HI	0.1-5.0

[a]Source: EPA, 1986.

Except for chlorination chemicals and the by-products they produce (e.g., chloroform), which are not the primary focus of this discussion, municipal water supplies are generally free of significant pesticide contamination. Isolated cases of municipal water supply contamination do occur. Most rural residents use either private wells or cooperative water supply and treatment facilities. Contamination of domestic wells by agricultural run-off is a potential problem, particularly in regions with sandy loam soils. The USDA estimated that over 17,000 people obtain drinking water from private wells in potentially contaminated areas (Nielson and Lee, 1987). Most of these reside on the eastern coast of the United States.

Current EPA National Primary Drinking Water Regulations apply to only a few chlorinated insecticides and herbicides. The EPA has proposed extending the list of regulated pesticides to 14 (EPA, 1985c), with tolerances for adults ranging from 0.42 mg/liter/day for aldicarb to 52 mg/liter/day for alachlor. According to the Safe Drinking Water Committee of the National Research Council, the average adult consumes 1.63 liters of water daily (NRC, 1977). Therefore, if pesticides were present in drinking water at these levels, water would represent the major exposure medium. Fortunately, this does not appear to be the case.

In Springfield, two municipal water samples showed low levels of *o*-phenylphenol (0.04 and 0.11 μg/liter) and one contained propoxur at 0.03 μg/liter. At these levels, daily intakes would amount to 0.1 to 0.5 μg for an adult. Municipal drinking water supplies in Bayonne and Elizabeth, New Jersey were monitored for *p*-dichlorobenzene during 1981-83 and positive findings were reported (>0.1 μg/liter) in only 2% of the samples (Wallace, 1987).

Drinking water is also included as one of the food commodities collected and analyzed by the FDA Total Diet Study. Few, if any, pesticide residues are found in these samples, which are collected from 21 districts across the United States (P. Lombardo, U.S. Food and Drug Administration, personal communication, 1988).

Diet Most home gardeners use pesticides, primarily insecticides, on the food they grow. While the variety of pesticides used by home gardeners is comparatively limited, it is not possible to assess the relative residue levels which may be found in home-grown food compared to that bought in the market place. Home gardeners may not follow proper application instructions, and residues may not be efficiently removed by usual washing procedures. For the average U.S. resident, however, the total quantity of pesticides ingested from commercial food sources probably outweighs that received from home-grown produce.

Prepared and stored food may also become contaminated from pesticides used within the home. This may occur from spray deposition during treatment (Jackson and Wright, 1975), or by diffusion of pesticide vapors into packaged and open foodstuffs stored in kitchen cabinets and pantries (Yeo, 1969). The relative importance of such contributions to food contamination is not known.

For the purpose of comparing the importance of the relative routes of exposure to pesticides used around the household, information collected by the Food and Drug Administration in its Total Diet Surveys may be used. Using data collected by the FDA from eight market baskets analyzed between April 1982 and April 1984 and assuming an average breathing volume to 20 m^3/day, some preliminary estimates of food intake vs. inhalation exposure has been made for participants of the spring 1987 NOPES program (Immerman and Firestone, 1989). In Jacksonville, Florida, food was estimated to be the primary source of exposure for 13 pesticides and inhalation for 8. However, inhalation accounted for over 60% of the total exposure of 35 μg/day. Corresponding numbers for Springfield, Massachusetts were 17 and 5, with only 36% of the total exposure of 22 μg/day attributable to the air route. Data on the 10 pesticides responsible for the highest estimated total exposure are given in Table 2-14. It should be noted that several of the most important pesticides found in air--*o*-phenylphenol, propoxur, and dichlorvos--are either not monitored in food or not found by the FDA Total Diet Surveys. It is likely that inhalation is the major exposure route for these pesticides. Since summertime levels are substantially higher than those in the spring, the relative contribution of inhalation exposure should be increased in summer.

Accidental and Intentional Ingestion Most pesticides present the risk of acute poisoning if ingested in either concentrated or diluted forms and both accidental and intentional (suicide) exposures occur. In most cases, ingestion is accidental and results from improper storage, labeling or use. Few, if any, of the common household pesticide products come in "child-proof" containers. Rodenticides and snail and ant baits, such as dicumarol, zinc phosphide, arsenates, metaldehyde, and strychnine also present poisoning hazards to small children and pets if used in areas accessible to them. Emulsifiable concentrates mixed with water have been mistaken for milk and have resulted in fatal poisonings even of adults (Lores et al., 1978; Maddy and Edmiston, 1988; Richmond, 1987). In Los Angeles alone during 1986, there were 2,441 reported calls involving children under five years old (Maddy and Edmiston,

TABLE 2-14. Estimates of Relative Contributions of Air and Diet to Exposure to Pesticides Commonly Used Around the Household, Spring 1987

	Jacksonville, Florida Mean Intake (μg/day)				Springfield, Massachusetts Mean Intake (μg/day)			
	Air[a]	Diet[b]	Total	% Air	Air[a]	Diet[b]	Total	% Air
Malathion	0.20	4.70	4.90	4	0.01	4.63	4.64	<1
Propoxur	4.40	--	(4.40)[c]	(100)	0.60	--	(0.60)	(100)
Chlordane	3.81	0.07	3.88	98	5.06	0.07	5.13	99
Chlorpyrifos	3.66	0.14	3.80	96	0.15	0.14	0.29	52
Heptachlor	2.63	<0.01	2.63	100	0.64	<0.01	0.64	100
Diazinon	2.25	0.59	2.84	79	0.20	0.59	0.79	25
Dichlorvos	1.80	--	(1.80)	(100)	<0.20	--	(<0.20)	(100)

TABLE 2-14. Estimates of Relative Contributions of Air and Diet to Exposure to Pesticides Commonly Used Around the Household, Spring 1987 (Continued)

	Jacksonville, Florida Mean Intake (μg/day)				Springfield, Massachusetts Mean Intake (μg/day)			
	Air[a]	Diet[b]	Total	% Air	Air[a]	Diet[b]	Total	% Air
Carbaryl	0.02	1.68	1.70	1	<0.01	1.71	1.71	<1
o-Phenylphenol	1.40	--	(1.40)	(100)	0.80	--	(0.80)	(100)
Captan	0.01	1.37	1.38	<1	<0.01	1.47	1.47	<1

[a] Based on 24-hour mean air concentrations determined with personal air samplers times 20 m^3 inhaled air/day.
[b] Dietary intake based on 1982-1984 FDA Total Diet Survey, adjusted only for average age of populations studied.
[c] Numbers in parentheses are estimates based on no dietary contribution.

1988). Nearly 40% of these occurred indoors and many involved accidental or suicidal ingestion.

Organophosphate and carbamate pesticides, which generally have high acute toxicities to man and domestic animals, are most frequently involved in poisoning by ingestion. Nearly all pesticides are poisonous to man, however, and should never be stored inside the home. The recognition and treatment of pesticide poisonings have been described in an EPA report (Morgan, 1977).

EXPOSURES OF BYSTANDERS TO PESTICIDE APPLICATIONS

Many persons who are not involved in pesticide application are exposed to pesticide residues in the workplace. A common place of exposure is in an office building or school after a pesticide application has been made. Inhalation and dermal exposure can lead to mild and serious illnesses. The cause is often a failure to provide several hours of ventilation with outdoor air. Studies have quantified air levels and horizontal surface levels of pesticide residues subsequent to such applications both with and without ventilation. Poison information records in California confirm these are common occurrences that can lead to acute poisonings. The rarity of repeat exposures to the same individuals indicate these exposures should not lead to chronic effects.

Agricultural field workers commonly experience two types of exposure to pesticides even though not involved in the applications. The first is drift of a pesticide during application in a nearby field. For a given individual these are unusual exposures, but they may lead to acute poisonings. The more common exposure to field workers involves reentering a treated field at some time after the application process. Reentry (waiting) periods are specified by regulatory agencies to reduce the likelihood of sufficient exposure occurring that would lead to acute poisoning.

Regulatory agencies are just beginning to impose reentry restrictions for reducing exposure to field workers that might be expected to lead to chronic effects. Much more work is needed in this area.

METHODS OF ASSESSING EXPOSURE TO PESTICIDES

Ambient Monitoring

Historically, pesticide exposure has been estimated using ambient monitoring methods (Bristol et al., 1984; Durham and Wolfe, 1962), that measures the amount of chemical that impinges on the body surface (skin, lungs, gastrointestinal tract). A significant deficiency in exposure estimates obtained from ambient monitoring is that they estimate the amount of pesticide reaching the body surface (contact exposure) and not the amount actually absorbed into the body (the dose) (Franklin et al., 1981).

Inhalation Exposure. Air measurements can be classified as either real-time or integrative (over a period of exposure). Except for relatively high concentrations (occupational levels), real-time measurements are difficult or impossible to make. Consequently, most air route exposure assessments are based on integrated measurements. Air sampling methodology for pesticides has been reviewed in depth (Lewis, 1976a; van Dyk and Visweswariah, 1975). It typically involves the collection of pesticides from air onto a filter, sorbent trap, or combination of the two, followed by extraction and chemical analysis.

Samples may be collected over 24-hour periods or for shorter periods of exposure time, depending upon the design of the study and the sensitivity of the method. When the usual gas or liquid chromatographic analysis procedures are used, air volumes of 0.01 to 1 m^3 are sufficient for occupational exposure levels (i.e., 0.1 to 10 mg/m^3) and 1 to 10 m^3 for non-occupational exposures (i.e., 0.01 to 100 $\mu g/m^3$).

Methods for several pesticides at occupational levels in air are given in the NIOSH Manual of Analytical Methods (Eller, 1984). An indoor air sampling and analysis method for a broad spectrum of pesticides at concentrations in the 0.01 to 50 $\mu g/m^3$ range has been recently published by the American Society of Testing and Materials (ASTM, 1988). For the very low levels (<1 ng/m^3) that may be found in ambient outdoor air, EPA Method T04 may be used (Riggin, 1984).

For best exposure estimates, personal air monitors are recommended. These are usually small sorbent traps attached to battery-powered pumps weighing 0.2 to 1.2 kg that can be worn or carried by the subject under study. These systems have been described in the literature (e.g., Coker, 1981; Lewis, 1976b; Lewis and MacLeod, 1982; Lewis and Wallace, 1989). They should be quiet in operation and easy to wear or transport. Sampling intakes are most often positioned in the "breathing zone," but are probably best placed where they are not directly influenced by exhaled air. Attachment of the sorbent trap or inlet, oriented downward, to the clothing at the back of the neck is a good practice in most situations. However, the wearing of personal sampling pumps is generally not convenient for most participants in non-occupational studies, especially children. In such cases, the sampler may be kept close by on a table, desk, or countertop, during which time it may be operated by means of an AC-to-DC power converter/charger. For monitoring periods longer than 24 hours, the latter procedure will usually be necessary due to limited battery life and to cover the sleep period.

Fixed-position area monitoring (e.g., outdoors, workrooms, living rooms, or bedrooms) combined with activity logs may also be used to estimate exposure. The portable pump-based systems used for personal exposure monitoring are also most convenient for area monitoring, provided that sufficient air volumes can be obtained. Intakes should be positioned 1 to 2 m above the floor or ground and oriented downward or horizontally to prevent contamination by non-respirable dustfall. If two or more samplers are to be used for collocated sampling, intake should be at least 30 cm apart for low-volume samplers (1 to 5 liters/min) and 1 to 2 m apart for high-volume samplers (up to 1000 liters/min).

Sampling media that have shown to be efficient for collection of multi-class pesticides from air are polyurethane foam (Billings and Bidleman, 1980; Lewis et al., 1977; Orgill et al., 1976; Wright and Leidy, 1982), Chromosorb^R 101 (Mann et al., 1974); Chromosorb^R 102 (Hill and Arnold, 1979; Thomas and Seiber, 1974); Amberite^R XAD-2 (Billings and Bidleman, 1983; Farewell et al., 1977; Jackson and Lewis, 1980; Johnson et al., 1977); Amberlite^R XAD-4 (Woodrow and Seiber, 1978); Tenax^R-GC (Billings and Bidleman, 1980, 1983; Jackson and Lewis, 1980; Lewis and MacLeod, 1982); Poropak^R-R (Jackson and Lewis, 1981), and Florisil^R (Jackson and Lewis, 1980; Yule et al., 1971). These sorbents appear to be about equally efficient for trapping most pesticides. Polyurethane foam (PUF) has enjoyed the most widespread popularity because it is more convenient to use and has much less resistance to air flow than the granular sorbents. However, a few of the more volatile pesticides may not collect efficiently on PUF.

Except for herbicide salts and a few other non-volatile compounds, most of the pesticides will either be present in air primarily in the vapor phase or will volatilize from airborne particulate matter readily after collection on a filter. Solid sorbent beds will collect most particulate-associated pesticides along with vapors; however, recent evidence suggests that some penetration of fine particulate matter (0.1 to 1 μm) may occur with PUF and Florisil (Kogan et al., 1988). Fine particles were not found to penetrate XAD-2 beds, presumably due to their retention by static charge. It may be good practice, therefore, to use a particle filter in front of the sorbent bed. In this case, the filter and sorbent bed should be extracted together for analysis to provide for better detection and prevent misinterpretation of the analytical results with respect to original phase distributions.

Dermal Exposure The patch technique has been the most widely employed method for estimating dermal exposure since its formal publication by Durham and Wolfe (1962). Methodology has been reviewed by Davis (1980) and Nigg and Stamper (1985). It is now an approved protocol for both the World Health Organization (Toxbettero 1986) and EPA (1986). However, there are many investigators who consider that the patch technique may be inadequate (Bonsall, 1985; Franklin et al., 1981; Lavy and Mattice, 1985). This conclusion is based on lack of correlation with urinary metabolite data and questionable accuracy of the patch data (Fenske, 1987). A new approach using a fluorescent tracer technique coupled with quantitative video imaging may provide more accurate estimate of dermal exposure (Fenske et al., 1985, 1986a, 1986b). There is a need for further research before this method can be more widely accepted.

Oral Exposure Methods for determination of pesticide residues in food are published by the Food and Drug Administration (FDA, 1988). Under the FDA Total Diet Study (Pennington and Gunderson, 1987), food commodities (including drinking water) were collected by inspectors of each of 21 district offices across the United States and prepared in an institutional kitchen to simulate diets of specific age groups of both sexes. These diets are analyzed for several groups of pesticides and the results are published in the Journal of the Association of Office Analytical Chemists (e.g., Gartrell et al., 1986a, 1986b).

Methods for monitoring drinking water supplies are discussed by Suffet and Malaiyardi (1987) and general guidelines have been published by EPA in the Federal Register (EPA, 1984, 1985a). Water is generally collected in 1- to 4-L samples from taps or wells for analysis according to methods published in the Federal Register (e.g., methods 608, 617-648, and 680). Methodology that is more appropriate for drinking water has been written as part of the National Pesticide Ground Water Survey and is available from EPA (1988).

Estimation of Dose Using Ambient and Biologic Monitoring

The concentration of chemical in the air can be used to estimate contact exposure to the lungs by multiplying it by the breathing rate. Empirically determined respiratory rates for adults are given in Table 2-15. From visual

TABLE 2-15. Normal Respiratory Rates for Humans[a]

Level of Activity	Respiratory Rate in Liters per Minute			
	Adult Avg.	Male Range	Adult Avg.	Female Range
Rest	7	6-10	4	4-7
Light Work	29	27-31	16	16-17
Heavy Work	60	50-90	24	17-32

[a] Data from Spector (1956).

observations, Wolfe estimated respiratory rates for agricultural and pesticides formulation plant works to range from 10 liters/min for females driving tractors to 42 liters/min for males performing heavy work (Lewis, et al., 1980). Standard physical parameters used by the EPA Office of Pesticide Programs for exposure estimates include breathing rates of 20 liters/min for males or 16.7 liters/min for females performing light work and 30 liters/min and 25 liters/min, respectively, for heavy work (Jensen, 1984). For non-occupational exposures, a 24-hour average of 10 liters/min is commonly used (Reinert, 1984), as is 20 m^3/day (13.9 liters/min) (EPA, 1985). Either of the latter two parameters should be acceptable (for adults) within the experimental error of monitoring measurements at non-occupational levels.

The collection of adequate quantities of household dust for analysis in a manner that will not jeopardize the integrity of the pesticide residues requires a special sampling device. Until recently, standard vacuum cleaners were typically used. However, with this manner of sampling the collected dust is subjected to continuous air flows that may result in evaporative losses of many pesticides. A sampler has been recently developed, and is now commercially available, which contains a cyclone, fine particulate filter, and polyurethane foam sorbent trap to assure total collection of house dust and the pesticide residues associated with it (Roberts et al., 1987b). It can collect 2 to 4 grams of dust from carpeted floors in 7 to 10 minutes. This sampler has been used in EPA's Non-Occupational Pesticides Exposure Study.

Exposure of users of pesticides which have the potential for causing adverse helath effects must be measured accurately in order to make meaningful risk determinations. Acceptable methodology is usually available to measure inhalation exposure; however, most pesticide exposure is dermal. Analysis of residues on absorbent pads placed on various parts of the body during exposure and subsequent extrapolation of dermal contact exposure may lead to an overestimate of dermal exposure. Availability of dermal absorption-rate data in rodents is useful, but the primate or guinea pig tend to provide a more accurate estimate of human absorption. The most useful data result from development of metabolism data in humans or a suitable test animal with appropriate pharmacokinetics, including excretory data, and then monitoring blood, urine, saliva, or feces of persons being exposed to the chemical under normal use conditions.

A pesticide safety program requires the presentation and consideration of data on: 1) pesticide vapors, mists, or dusts in the breathing zone of exposed persons; 2) pesticide dusts, powders, or liquids on the skin of persons mixing, loading, and/or applying pesticides; 3) pesticide residues, including the more toxic breakdown products, on foliage and in soil of fields where work is to take place which may later contact skin; and 4) residues in the air, on floors, counters, furniture, etc., following application of pesticides indoors.

These measurements are of value in designing methods to reduce exposure to persons agriculturally exposed, such as commercial agricultural applicators, but also to persons who use pesticides in non-agricultural settings.

In the context of *biological monitoring*, data collected on dermal deposition with current methods, when used along with the animal dermal absorption rate or dermal dose-response data, are often assumed to overestimate actual exposure. Metabolism data are used to determine pharmacokinetic and excretion parameters and can form the basis for biological monitoring. For example, if a parent chemical or a urinary metabolite can be fully characterized and tested for, then the amount of the chemical in the urine of users can be monitored as a measure of internal dose. At the same time, the amount of chemical that might be inhaled and the amount that falls on the skin can also be measured. With such data, a much more objective assessment can be made of the actual exposure. Use of such data may indicate safe use even though dermal deposition data and the animal study dermal absorption rate data alone might signal an unacceptable risk.

Increased emphasis on occupational health hygiene and safety led to further development of human monitoring to supplement or replace ambient monitoring (Ashford et al., 1984). Biological monitoring has been defined as the analysis of the chemical, its metabolite or biotransformation products in body tissues such as urine, blood, fat, hair, nails, sweat, saliva and expired air (Berlin et al., 1979). A distinct advantage to using biological monitoring is that it provides an integrated measure of exposure from all routes and sources, allowing a more meaningful risk assessment to be made.

One way of using biological monitoring is to measure metabolites of the pesticide in urine to indicate exposure to that pesticide. This approach is useful to monitor workers who are exposed on a regular basis, and spot urine samples can be used to check exposure. Another use of biological monitoring is to measure pesticide levels in adipose tissue as has been done in the U.S. National Human Adipose Tissue Study.

Biological monitoring has been used in worker exposure studies to assess the reliability of the patch data and it appears that estimates of exposure from patches do not correlate all that well with the urine metabolite data (Fenske, 1989; Franklin et al., 1981; Lavy and Mattice, 1985). Efforts are underway to assess this discrepancy.

It may also be possible in the future to use the urinary metabolite data to estimate dose. However, the relationship between dose and toxic effect is dependent upon many factors: Uptake, distribution, metabolism and excretion all have a role in determining blood levels, concentration at target tissue, and urinary metabolite concentration. It would be simplistic to presume that the metabolite concentration could be corrected for molecular weight and used to estimate dose. One approach would be to obtain more data in animals and to measure urinary metabolite levels in the toxicity studies. This could allow a comparison to be made between the concentration of metabolite and the toxic effects and a metabolite/response curve to be drawn analogous to the dose/response curve. If it were known that the metabolism in humans was similar to the test animal, the urinary metabolite levels could be related to effects. It is obvious that considerable work has to be done to determine the feasibility of this approach.

Biological monitoring can also include the measurement of a health effect. For example, measurements of cholinesterase enzyme levels have been used to monitor exposure to organophosphorus and carbamate pesticides. This procedure has limited use unless the program is carefully set up or unless the person has had a very high exposure.

Recently, Lotti (1989) has summarized work on the use of an enzyme (NTE) in circulating human lymphocytes as a marker for exposure to organophosphorus pesticides that induce delayed neurotoxicity. DNA adducts

(Shugart et al., 1989) and hemoglobin adducts are also possible methods for monitoring exposure.

CONCLUSIONS

1. **During their working life, pesticide applicators and associated workers may be exposed to pesticides in quantities measurable in milligrams per hour (mg/hr).**

2. **Harvesters may be exposed to amounts of pesticides similar to those encountered by applicators during their working life. In addition, they can be exposed to more toxic products resulting from environmental transformation of the pesticides applied.**

3. **Methodologies exist for measuring exposure of applicators by the inhalation and dermal routes.**

4. **Non-occupational pesticide exposure occurs at levels measurable in mg/day. This exposure is not necessarily continuous.**

5. **Children and adults of reproductive age are populations of special concern due to size and physiologic status.**

6. **Exposure to pesticides occurs from a variety of sources, including natural toxicants in foods. Exposure to synthetic pesticides through food products is usually extremely low or nonexistent.**

7. **Exposure to pesticides by the largest number of persons probably occurs in and around the home.**

8. **Of all pesticide exposures, pesticide residues in food are the most extensively monitored; however, research on a broader scale and with more rapid methods should continue.**

RECOMMENDATIONS

FDA monitoring data on residues in foods should be augmented by data existing in the private sector and other government organizations; a data management system should be created for its efficient use.

Carefully designed epidemiologic studies of human health effects of pesticide exposure of agricultural and non-agricultural workers should be conducted. Acute and chronic outcomes should be included.

A system should be established for reporting all agricultural and non-agricultural pesticide use by all occupational categories of applicators. All pesticide-related illness, both occupational and non-occupational, should be reported, including anecdotal reports. An annual nationwide report of summary statistics should be published. Pesticide registrants' reports of pesticide illness should be made quarterly and become a part of the annual report.

Re-entry intervals should be established for all pesticide use areas, including homes and lawns, based on acute, subchronic, and chronic toxicologic end points. Re-entry intervals should be based on models constructed with human monitoring experiments and validated with field experiments.

Exposure monitoring should be aggressively improved. Accordingly,

1. A concerted effort should be directed towards development of biologic methods to estimate internal dose in humans and in animals;

2. Methodology should be improved to estimate contact exposure and absorption of products for which biologic monitoring is inappropriate;

3. Methods to be used for biologic and ambient monitoring should be standardized and validated before registration of the pesticide. Emphasis should be given to developing reliable, inexpensive and non-invasive methods.

4. Consideration should be given to making applicator exposure studies a registration requirement. Optimally, intensive human biologic monitoring of a new pesticide should be required prior to the first U.S. registration. Such studies should first be conducted under permits for experimental use, simulating the usual conditions of pest control practices, and should continue for at least the first year of registered commercial use. Any adverse effect detected during actual use should be reported immediately and investigated;

5. The importance of the routes of pesticide exposure (oral, dermal, inhalation) for the general population should be vigorously examined experimentally. The study of indoor exposure should be emphasized. Exposure estimates should include information on exposure to inert ingredients.

- Qualitative and quantitative characterization of natural pesticides and toxicants in food plants should be made under both constitutive and induced circumstances.
- An evaluation of the dietary intake of these chemicals should be made.
- Earlier recommendations that the American public increase their intake of cruciferous vegetables should be reconsidered; recent studies have suggested that cruciferous vegetables may be promoters of carcinogenesis.

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CHAPTER 3

NEUROTOXIC EFFECTS OF PESTICIDES

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INTRODUCTION

The term "neurotoxicity" is used to describe the potential of an agent to alter structure or function of the nervous system as defined by indices of neurochemistry, neuropathology, behavior, and such specific psychologic processes as perception, motivation, habituation, learning, and memory. Many of the relevant indices can be measured directly by neurochemical, neurophysiologic, and neuropathologic techniques, whereas others must be inferred from observed behavior. The modes of action of many classes of pesticides--for example, the insecticides--would lead immediately to a consideration of potential neurotoxicity. With other classes of agents, the association with neurotoxicity could be much more subtle.

This chapter will present the known effects of pesticides on both humans and animals. The symptomatology associated with central nervous system (CNS) and peripheral deficits resulting from exposure to some organochlorine insecticides will be discussed. A section on anticholinesterases (organophosphorus and carbamic acid esters) will describe organophosphate-induced delayed neuropathy (OPIDN), the polyneuropathic effects of certain organophosphorus esters and carbamates on motor nerves, and the newly identified "intermediate syndrome." The toxic effects characteristic of some synthetic pyrethroid esters will be discussed. There is as yet little evidence to link herbicides, fungicides, and fumigants to impairment of the CNS in humans, but the effects of these agents on neurologic parameters in animal models will be described. In addition, the neurotoxic effects of pesticides in the developing organism will be addressed. Current regulatory status and concerns that need to be addressed in the assessment of neurotoxicity are considered.

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EPIDEMIOLOGY OF NEUROTOXIC EFFECTS

The toxicity of pesticides in humans has been extensively studied and clearly documented in a number of publications (Ecobichon and Joy, 1982a; Hayes, 1982). Having treated the immediate signs and symptoms of toxicity, attending physicians dismiss the apparently stabilized patient and subsequently have little contact with the individual. It has become obvious that, in many cases, this is not the termination of the poisoning. Well-documented cases and epidemiologic studies, as well as a large number of uncontrolled, nonquantitative, anecdotal presentations of incidents, suggest that (1) symptoms can persist for several years, and (2) some of the major symptoms are associated with peripheral or central nervous system toxicity. The symptomatology appears to be related to behavioral, psychologic, motor, sensory, autonomic, and cognitive difficulties and may arise subsequent to or in the absence of the classical signs of acute poisoning. The nature of the neurotoxicology is best illustrated by the following recently published case study, abbreviated here to describe only the chronologic development of the patient's condition (Branch and Jacqz, 1986a).

> The patient, a 75-year old male, was exposed continuously over a period of 8-10 months to carbaryl applied repeatedly (six times) as a 10% dust to the basement area of his house to control fleas. The central air conditioning, located in the basement, dispersed the insecticide dust throughout the structure. The entire family (husband, wife, son) experienced a range of influenza-like symptoms (headache, malaise, epigastric discomfort, abdominal colic, diarrhea, nausea, muscle spasms, cough) within three days of the initial application of the carbaryl dust. These symptoms were consistent with the early signs of poisoning by an anticholinesterase-type insecticide.
>
> Whereas, the symptoms of the wife and the son disappeared, those of the patient became progressively worse with time: increasing dyspepsia; muscle spasms that became severe enough to prevent him from driving a car; headaches associated with severe pressure sensation; tinnitus, vertigo and occasional mild ataxia, rhinorrhea and excessive lacrimation; weakness of major muscle groups and muscle fasciculations; somnolence; weight loss, agitation and confusion. The persistent symptoms resulted in a series of

> investigations, including repeated upper gastrointestinal series, barium enema, liver/spleen scanning, ultrasound examination, lung scanning, body computed tomographic scanning, intravenous polygraphic scanning and cysternography. All investigations were negative except that computed tomographic scanning revealed evidence of cerebral atrophy not observed in a scan prior to the carbaryl exposure.
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> The patient's wife noted the heavy deposit of white dust in the basement, and the presence of carbaryl was confirmed by a state government inspector. An analysis of plasma and erythrocytic cholinesterases revealed lower than normal activity of the plasma enzyme in the patient. The patient's symptoms improved while hospitalized. An intradermal allergy test to carbaryl did not provoke a skin reaction. The patient moved into a motel but, while supervising a more extensive cleaning of his home, he experienced a return of his symptoms. A routine physical examination revealed a markedly irregular pulse and, on re-admission to the hospital, he was shown to have a sinus bradycardia with multiple ventricular ectopic beats, in addition to mild weakness and a positive Romberg test (inability to maintain body balance when the eyes are shut and the feet are close together). In addition, the plasma cholinesterase activity was low. Once again, his clinical condition, including the cardiac irregularity, improved during hospitalization.
>
> On being discharged, the family moved out of the house and had all of their possessions cleaned. The patient's symptoms improved, although it was two months before the plasma cholinesterase level returned to normal range. His body weight slowly returned to normal and he recovered his mental acuity, muscle power and co-ordination. The muscle cramps and fasciculations abated as did the rhinorrhea and excessive lacrimation. Symptoms which failed to resolve included an alteration in sleep pattern with episodes of waking with a headache, tinnitus and a sense of confusion, these episodic occurrences persisting for a further two years after exposure. The final residual neurologic defect that has persisted and become more severe since the initial exposure is defined as a glove-and-stocking peripheral neuropathy.

The literature on carbaryl states that it is a reversible inhibitor of cholinesterases both *in vitro* and *in vivo* and that the signs and symptoms of poisoning can be mild to severe but usually are of short duration (Branch and Jacqz, 1986b). Carbaryl is extensively and rapidly biotransformed and degraded by hydrolytic and oxidative mechanisms in mammalian systems and elimination of degradation products from the body is rapid. However, it would appear to persist longer in the human than in some other species (Knaak et al., 1965; 1968). It is obvious from the above-mentioned case study that the patient experienced sustained and repeated exposure to a high concentration of carbaryl with reduced plasma cholinesterase activity temporally related to the onset of symptoms, with reversal of most symptoms during the periodic reduction in exposure. There is little evidence of such a pattern of symptomatology in the literature on human poisonings. Carbamates are not considered to be chronic neurotoxic agents. However, chronic carbaryl administration in pigs can elicit chronic physiologic and morphologic neuromuscular and neurologic effects unrelated to the acute reversible cholinesterase inhibition (Smalley et al., 1970). Only in swine has repeated exposure to carbaryl resulted in ataxia, difficulty in standing, uncoordinated movements, exercise-induced tremors, and chronic muscle contractions with concomitant micropathologic conditions being observed in the central nervous system and skeletal muscles (Smalley et al., 1968; 1970).

Case histories such as that described above, where exposure involved only one agent, constitute only a small fraction of the total number of individuals exposed to pesticides. Rarely will one encounter situations where a pesticide handler (manufacturer, formulator, agricultural sprayer, or structural sprayer) will be exposed to only one agent. The agriculturalist must use a wide range of pesticides to ensure maximum crop production and to control insects, weeds, fungi, etc. There will be seasonal exposure to a variety of chemicals over a number of months--from May to September in northern climates and from March to November in southern climates. Lawn and turf specialists will encounter a similar pattern of exposure. Greenhouse personnel experience a year-long exposure, as do structural applicators for pest control; exposure in these professions frequently takes place in enclosed and poorly ventilated areas. The manufacturer and formulator can be exposed to a variety of agents at relatively high levels as marketing needs are fulfilled for commercial users. Such exposures give rise to the following scenarios.

Acute Neurologic Effects from Acute Pesticidal Exposure

By virtue of their mechanism(s) of action on sensory and motor neurons, insecticides (organochlorines, pyrethroids, and organophosphorus esters and carbamates) are most likely candidates to elicit neurotoxicity. The published literature contains many examples of symptomatology of acute poisonings, but few reports of deaths, associated with occupational exposure (Edson, 1969; Namba et al., 1971; Morgan et al., 1980). Acute toxicity induced by insecticides is readily recognized and treated, and in most cases the biologic effects disappear with time. In most instances of pesticide-related deaths, there have been accidental or suicidal ingestions of moderate to large quantities of an agent or agents.

Chronic Neurologic Effects from Acute Pesticidal Exposure

It is apparent from a survey of published literature and anecdotal reports that acute exposure to insecticides can initiate long-term neurologic disabilities not observable at the time of acute signs and symptoms (Branch and Jacqz, 1986a; Ecobichon, 1982b; Joy, 1982a). This chronic neurotoxicity is not limited to insecticidal chemicals, however; it can also be associated with herbicides and fungicides, thereby complicating the situation for the occupationally exposed worker who may encounter a seasonal exposure to all three classes of pesticides (Agapejev et al., 1986). The acute, limited, annual exposure to such agents may elicit few discernible signs and symptoms on an annual basis but may contribute to neurologic deficits on a long-term basis (Davignon et al., 1965; Kashyap, 1986). A follow-up study of 114 pesticide workers detected optic, gastrointestinal, cephalagic, cardiorespiratory, and neuropsychiatric complaints that persisted for up to 3 years after organophosphorus ester poisoning (Tabershaw and Cooper, 1966).

Chronic Neurologic Effects from Chronic Pesticidal Exposure

An extension of the previous scenario, the annual exposure to a variety of pesticides over a period of 10 to 20 years can result in mild-to-severe deterioration in neurologic function. A very few extensive studies of selected agricultural workers have revealed that insecticides, in particular, may elicit chronic neurologic manifestations in man (Davignon et al., 1965; Kashyap, 1986). Davignon and colleagues found a positive relationship between the incidence of neurologic abnormalities (loss of reflexes, tremors, disturbance of equilibrium) and the number of years of exposure to organochlorine and organophosphorus insecticides. In certain cases, neurologic sequelae persisted for only a few months, discernible improvement being observed by frequent testing with batteries of psychologic tests administered during the postexposure period (Sidell, 1974; Ecobichon et al., 1977). In other situations, the neurologic problems have been known to persist for many years (deJager et al., 1981; Kashyap, 1986; Korsak and Sato, 1977; Spiegelberg, 1961). There is limited evidence that lifetime exposure to low levels of pesticides may be associated with the development of idiopathic Parkinson's disease in rural areas (Barbeau, 1984; Barbeau and Roy, 1985; Rajput et al., 1987).

A vast array of neurotoxic effects can be associated with pesticides. The most prevalent chronic symptoms observed following exposure to pesticides, particularly insecticides, have been lethargy, fatigue, a partial transient paralysis and/or weakness in the peripheral muscles in the hands and feet, all signs suggestive of a polyneuropathic action on the motor nerves and possibly the neuromuscular junctions (Petry, 1951; Petty, 1958; Ecobichon et al., 1977; Hierons and Johnson, 1978; deJager et al., 1981; Curtes et al., 1981; Vasilescu et al., 1984). In contrast to the classical organophosphate-induced delayed neuropathy of distal muscles in limbs caused by select agents (Ecobichon, 1982b; Johnson, 1982), other organophosphorus and carbamic acid esters have been associated with clinical and subclinical neuropathic signs that appear to be related to other mechanisms of neurologic damage (Branch and Jacqz, 1986b; Stahlberg et al., 1978). Recently, a new type of syndrome associated with organophosphorus esters, an "intermediate syndrome," has been described in which the clinical course is quite different (Davies, 1987; Senanayake and Karalliedde, 1987).

Peripheral sensory deficiency is a well-known neurotoxic consequence of exposure to some organochlorine insecticides, the tingling and/or numbness (paresthesia) on the exposed skin being associated with altered conduction in sensory nerves (Joy, 1982a). Recent papers have described similar effects following cutaneous contact with certain synthetic pyrethroid esters (Kolmodin-Hedman et al., 1982; LeQuesne et al., 1984; Tucker and Flannigan, 1983).

The organochlorine compounds, the organophosphorus esters, and the carbamates are known for their effects on the central nervous system (CNS), manifested in cognitive deficits with loss of memory, of skills, and the ability to learn and perform any but the most simple of tasks (Ecobichon and Joy, 1982; Ecobichon et al., 1977; Korsak and Sato, 1977; Levin and Rodnitzky, 1976; Metcalf and Holmes, 1969). Such parameters as forgetfulness, mental confusion, difficulty in performing "thinking" tasks, irritability, recent memory loss, altered sleeping habits, linguistic disturbance, nervousness, agitation, and anxiety all belong to a catalog of effects related to CNS impairment. It should be emphasized that the last three signs are not unique; they would be symptoms anticipated in any poisoning situation.

From the occupationally exposed individual to the bystander, a wide variety of neurotoxic signs might be anticipated. It will be possible to identify and associate more overt forms of sensory and motor neurotoxicity; however, little effort has been devoted to investigation of the subtle neurologic changes associated with chronic sensory, motor, cognitive, and behavioral disabilities. For example, the contribution of herbicides, fungicides, and fumigants to the impairment of the CNS cannot be readily evaluated because of the complex mixture of agents to which many workers are exposed (Agapejev et al., 1986). Particularly difficult will be the assessment of possible neurologic effects associated with a lifetime exposure to trace amounts of various pesticides acquired in the daily diet. In such situations, neurologic changes may not be dramatic enough to be detected with the methodology in use today.

POTENTIAL FOR NEUROTOXIC EFFECTS

Organochlorine Insecticides

Representative organochlorine insecticides are shown in Table 3-1, separated into three groups based upon their acute and chronic toxicity in humans. DDT and its derivatives all produce similar toxic effects that differ in important ways from those produced by the other groups. The hexachlorocyclohexanes and the cyclodienes constitute a second group of organochlorines with similar toxicities. Exposure to chlordecone affects humans in a manner that differs significantly in many ways. A number of important reviews are available that have examined the consequences of human and animal exposure to these insecticides (Ellenhorn and Barceloux, 1988; Gupta, 1976; Guzelian, 1982; Hayes, 1959a; 1975; 1982; Joy, 1982a; Morgan, 1982; Woolley, 1982). They should be consulted for more detailed information about the acute and chronic effects of these chemicals in both humans and animals.

DDT and Its Derivatives Exposure to DDT or to its derivatives can result in an acute toxic response. Very few deaths have been reported. After ingestion, the earliest effects involve paresthesias of the mouth and face. Altered motor function leading to ataxia and abnormal stepping reactions also tends to occur early. These symptoms are typically followed by dizziness, confusion, general malaise, vomiting, headache, and fatigue. Tremor, particularly of the hands, is a common symptom of DDT poisoning. Convulsions have been rarely reported and result from ingesting large quantities of DDT. Onset of symptoms may be as early as 30 minutes after ingesting large doses or may be delayed as much as 6 hours after ingesting smaller amounts. Dermal and inhalational exposures have rarely been reported to result in measurable neurotoxic effects. In the vast majority of exposure reports, if any symptoms were observed at all, recovery was usually complete within 1 to 3 days (Hayes, 1982). Symptoms such as weakness and fatigue were reported to continue for weeks after a single exposure (Garrett, 1947).

Chronic exposure to DDT may result in the delayed emergence of symptoms that develop slowly and show certain additional complications not part of the acute poisoning syndrome (i.e., loss of weight, anorexia, mild anemia, muscular weakness, and tremors). Anxiety, nervous tension, hyperexcitability, and fear are commonly expressed by the affected person. Electroencephalographic abnormalities may be encountered even when no

TABLE 3-1. Signs and Symptoms of Acute and Chronic Toxicity Following Exposure to Organochlorine Insecticides

Insecticide Class	Acute Signs	Chronic Signs
Dichlorodiphenylethanes		
DDT	paresthesia (oral ingestion)	loss of weight, anorexia
DDD (Rothane)	ataxia, abnormal stepping	mild anemia
DMC (Dimite)	dizziness, confusion, headache	tremors
Dicofol (Kelthane)	nausea, vomition	muscular weakness
Methoxychlor	fatigue, lethargy	EEG pattern changes
Chlorbenzylate	tremor (peripheral)	hyperexcitability, anxiety
	nervous tension	
Hexachlorocyclohexanes		
Lindane (gamma isomer)		
Benzene hexachloride (mixed isomers)	dizziness, headache	headache, dizziness
	nausea, vomition	hyperexcitability
Cyclodienes	motor hyperexcitability	intermittent muscle twitching and myoclonic jerking
	hyperreflexia, myoclonic jerking	
Endrin	general malaise	psychologic changes including insomnia, anxiety, irritability
Telodrin	convulsive seizures	

TABLE 3-1. Signs and Symptoms of Acute and Chronic Toxicity Following Exposure to Organochlorine Insecticides (Cont.)

Insecticide Class	Acute Signs	Chronic Signs
Cyclodienes (Cont.)		
Isodrin		
Endosulfan		EEG pattern changes loss of consciousness epileptiform convulsions
Heptachlor		
Aldrin		
Dieldrin		
Chlordane		
Toxaphene		
Other		
Chlordecone (Kepone)		chest pains, arthralgia skin rashes ataxia, incoordination, slurred speech, opsoclonus visual difficulty, inability to focus and fixate nervousness, irritability, impairment of recent memory, depression muscle weakness, tremors in hands severe impairment of spermatogenesis
Mirex		

neurologic or clinical evidence of abnormality is seen (Mayersdorf and Israeli, 1974). However, such reports are rare, even among populations involved in manufacture, formulation, or spraying. In many cases, no symptoms have been reported or observed in workers using DDT without special precaution even though they had blood levels of DDT 3 to 15 times higher than control subjects (Hayes, 1982).

Hypersensitivity to DDT and related compounds has been suggested (Kailin and Hastings, 1966). Such reports are unusual, and it is doubtful that DDT is any more likely to cause hypersensitivity responses than any other chemical. Occasional reports have associated DDT, alone or in combination with one or more other ingredients, with polyneuropathic phenomena (Campbell, 1952; Jenkins and Toole, 1964; Onifer and Whisnant, 1957; Stone and Gladstone, 1951; Wigglesworth, 1945). Considering the previous extensive use of DDT and its relatives, and the presence of other chemical substances in these cases, the likelihood that DDT is the causative factor is extremely low. Since 1972, the U.S. general body burden of DDT and its metabolites has dropped more than 50 percent (Davies and Lee, 1987). There is no compelling evidence available to suggest that exposure to DDT or its congeners at levels which exist in the U.S. environment today has any deleterious neurologic or behavioral effects.

Studies in animals have indicated that most species respond similarly to humans when acutely or chronically exposed to DDT. The mechanism by which DDT and its derivatives produce their effects, at least upon acute exposure, has been reasonably well defined. DDT causes repetitive firing in sensory and motor nerves. DDT does this by prolonging the falling phase of the action potential (Narahashi and Yamasaki, 1960; Welsh and Gordon, 1947). The prolongation results from a delay in sodium channel inactivation (Narahashi and Haas, 1967; 1968). The persistence of sodium conductance may continue for many milliseconds (Hille, 1968). Repetitive discharge of nerve fibers increases synaptic activity, and this is presumed to account for many of the central and peripheral effects of DDT (Joy, 1982a). Various models of the "DDT receptor" have been proposed. Good reviews of these have been provided by Metcalf (1973) and Woolley (1982).

Animal studies have shown that DDT can affect neurologic and behavioral function, although very few studies have actually been performed. Animal tests for the measurement of specific behavioral functions are available and need to be used more extensively than they have. Exposure to DDT can produce demonstrable changes in locomotion in rats (Khairy, 1959). Mice exposed to DDT during gestation and weaning, at a level lethal to 40 percent of the offspring, had impairments in learning and retention of a maze task (Craig and Olgilvie, 1974). Exposure of rats to 100 mg/kg DDT immediately after passive avoidance training impaired retention of the behavior 7 days later (Tilson et al., 1987). DDT also increased acoustic startle responsiveness of rats (Saitoh et al., 1986). The extrapolation of these data relative to the consequences of low-level DDT exposure on human personality, emotionality, learning, and memory remain to be established.

Hexachlorocyclohexanes and Cyclodienes Lindane, the gamma isomer of hexachlorocyclohexane, is an insecticide that shares a similar spectrum of action and toxicity with the cyclodienes. Benzene hexachloride (BHC) is the technical grade of hexachlorocyclohexane and contains five different isomers. The primary active ingredient of BHC is lindane. Various batches of BHC contain between 8 and 15 percent of the gamma isomer. These chemicals will be considered along with the cyclodienes in this section.

The typical response to the ingestion of toxic amounts of lindane or a cyclodiene insecticide is the development of convulsive seizures. These are often sudden and abrupt in onset. In some cases, they may be preceded by signs of motor hyperexcitability such as hyperreflexia and myoclonic jerking, but they may also occur without warning or any prodromal symptoms. Sensory disturbances are usually minimal. Headache, nausea, vomiting, general malaise, and dizziness may or may not be experienced. This occurs most frequently with the highly toxic and not very persistent compounds. The majority of endrin poisonings (Coble et al, 1967; Davies and Lewis, 1956; Weeks, 1967) and lindane poisonings (Eskanazy, 1972) described in the literature fit into this category. The cases described by Coble et al. (1967) are remarkable in that some of the victims were not even aware that they had had a convulsive episode. In one case, a woman was brought to the hospital because of a tender lump on her forehead. She had no explanation for it and could not remember how it got there. Shortly thereafter she had a generalized convulsion lasting about 2 minutes. Poisoning on an epidemic scale has also been reported. Weeks (1967) described four outbreaks of acute poisoning occurring in Qatar and Saudi Arabia. Altogether, 874 persons were hospitalized and 26 died. In all four situations, the cause of poisoning was determined to be flour that had become contaminated with endrin.

With compounds having a lower acute toxicity but a greater persistence in the body, cumulative intoxication can occur. Clinically, this results in a syndrome of headache, dizziness, drowsiness, hyperirritability, general malaise, nausea, anorexia, and occasional vomiting. At times, muscle twitchings, myoclonic jerks, and convulsions occur. Convulsions are particularly apt to develop suddenly and precipitously if the intensity of exposure is suddenly increased. This type of intoxication is typical of many poisonings reported with aldrin, dieldrin, and telodrin.

The description by Hayes (1957) of toxicity occurring in spraymen applying dieldrin exemplifies the consequences of chronic exposure to relatively small daily amounts of dieldrin. In certain pest control programs carried out in South America, 10 to 20 percent of spraymen had been poisoned. The earliest observed signs of poisoning occurred after about 3 months of exposure, but in the majority of cases, exposures of 8 months or longer were required. The mildest clinical illnesses were characterized by headache, blurred vision, dizziness, slight involuntary movements, sweating, insomnia, nausea, and general malaise. More severe illness was characterized by these same symptoms plus myoclonic jerking involving one or more limbs, sometimes resulting in the patient falling to the ground. In some cases, the myoclonic movements were accompanied by momentary loss of consciousness. In the most severe cases, one or more epileptic convulsions occurred. Convulsions were reported by over half of those patients who sought medical aid. One sprayman had more than 30 convulsions.

The presence of convulsions is a particularly disturbing consequence because convulsions, regardless of origin, can directly cause neuronal cell death and persisting neurologic symptoms. In spraymen studied by Hayes (1957; 1959b), symptoms began to subside upon removal from exposure, and most symptoms had disappeared within a few days. In some individuals, particularly in those having severe or multiple convulsions, persisting symptoms were observed. In a separate instance, members of a small Indian village community accidentally ingested grain mixed with aldrin dust and lindane powder for 6 to 12 months (Gupta, 1975). Many developed repetitive myoclonic jerking and other evidence of convulsive behavior. In some, symptoms persisted for over a year after exposure was terminated (Gupta, 1975). Mental retardation and hyperkinetic behavior were noted in two children (prior history unknown) who ate aldrin and lindane for 6 months. In a retrospective study of poisoning in children (Angle et al., 1968), acute poisoning by an organochlorine insecticide was the single most frequent cause of toxic convulsions. Although the sample size was small, their study showed an association of toxic convulsions, particularly those due to organochlorine insecticides, and later intellectual deficit. It seems clear that persisting sequelae can follow exposures to these insecticides when they produce convulsions.

It is less clear that persisting sequelae are likely to follow chronic exposure in the absence of convulsions. Changes in the EEG occur during intoxication, and some degree of abnormality may persist for weeks or months (Jager, 1970). The great majority of abnormalities described are bilateral and symmetrical and are reminiscent of changes described for myoclonic, photomyoclonic, and certain petit mal variant epilepsies. In cases where adequate preexposure EEGs were available, it has been estimated that normalization may require up to 2 to 3 months for endrin, a year for dieldrin, and more than a year for telodrin. The thorough study by Jager (1970) of insecticide-related toxicity occurring at the Shell Nederland Chemie in Rotterdam, the Netherlands, between 1954 and 1970, found that symptoms persisted in accordance with the time required for blood levels to fall. Thus, workers intoxicated by telodrin complained of persisting symptoms for periods up to 6 months, whereas workers poisoned with endrin or dieldrin were symptom-free after a few weeks. EEG changes outlasted reports of objective symptoms on the part of the patient and outlasted any clinical or laboratory findings.

Isolated cases have suggested that, on rare occasions, these chemicals may precipitate a peripheral neuropathy. Schuettmann (1972) observed polyneuritis in workers exposed to lindane. Motor pathology and psychologic disorders including anxiety, irritability, and insomnia were also detected. Latent polyneuropathies have been related to exposure to a combination of DDT and lindane (Onifer and Whisnant, 1957) or a combination of aldrin and DDT (Jenkins and Toole, 1964). Considering the level and frequency of exposure by people to these substances, their propensity to produce polyneuropathies appears to be very small.

Studies in animals have replicated the acute and chronic effects of exposure to these compounds. Most species exhibit reactions to acute and chronic exposures similar to the reactions of humans. A mechanism proposed to be responsible for many of the effects of these insecticides is the antagonism of GABA-mediated inhibition in the central nervous system. Lindane and the cyclodienes bind to a site on the GABA receptor-chloride channel complex (Abalis et al., 1985; Casida and Lawrence, 1985; Eldefrawi et al., 1985; Lawrence and Casida, 1984; Matsumura, 1985; Matsumura and Ghiasuddin, 1983). They also inhibit GABA-induced chloride flux in mammalian brain preparations (Abalis et al., 1986; Bloomquist et al., 1986; Bloomquist and Soderlund, 1985; Gant et al., 1987). Lindane produces an apparently selective antagonism of GABA-mediated inhibition in the mammalian hippocampus. Its effects at that locus are indistinguishable from the known GABA antagonists, picrotoxin and bicuculline (Joy and Albertson, 1987a; 1987b; 1988).

Most of the limited data base on the effects of these substances on behavior has been evaluated (Joy, 1982a; 1982b). It was concluded that (1) the capacity of these substances to modify behavior is dose dependent, (2) performance on complex behavioral tasks is more readily disrupted than performance on simpler tasks, and (3) transitional behaviors, such as occur during acquisition or extinction of responses, are much more susceptible to disruption than are steady-state behaviors. Little data are available on low-level, long-term exposures. Both lindane and dieldrin potentiate kindling, an experimental model of brain plasticity at low levels of exposure (Joy et al., 1980; 1982). Exposure of rats to lindane before birth or until weaning increases their susceptibility to kindling when adult (Albertson et al., 1985). More extensive studies of the effects of low-level, long-term exposures need to be done.

Chlordecone One of the worst incidents of known U.S. industrial exposure to chemical substances involved chlordecone. In 1975, some 133 individuals were poisoned while employed during a 17-month period of chlordecone synthesis under very unsatisfactory hygienic and health conditions. The "Hopewell epidemic" was associated with the discharge of large quantities of chlordecone into the air and into the local sewage system. Chlordecone eventually contaminated the James River and the tidal areas of the southern end of the Chesapeake Bay.

Patients with prolonged exposure to chlordecone developed nervous-ness, tremors, chest pains, loss of weight, arthralgia, skin rash, mental changes, opsoclonus, muscle weakness, ataxic gait, incoordination, and slurred speech (Taylor et al., 1978). In severe cases, the tremor was present at rest and, in all cases, was increased by use of the affected limb. Although the hands were primarily involved, trembling of the entire body (called "Kepone shakes") was also observed. A prominent complaint was visual difficulty, characterized by an inability to fixate and focus. Personality changes were recorded, the most common being irritability, difficulty with recent memory, and depression. A severe impairment in spermatogenesis was seen in a number of the men.

The average latency between start of exposure to chlordecone and onset of symptoms was 6 weeks. Severity of neurologic symptoms was proportional to the "dose" and duration of exposure. Approximately 50 percent of the total work force investigated was moderately ill and a few required hospitalization for several months. In the subsequent 18 months after detection, most symptoms and signs gradually disappeared but, even after 4 years, several workers continued to manifest a mildly incapacitating tremor (Taylor 1982). A subsequent follow-up of the 23 most severely affected patients between 1980 and 1982 (Taylor, 1985) indicated that symptoms persisted in seven cases. Five of these complained of persistent tremor or nervousness. Three stated that their tremor was present only when they were upset or angry, while the other two complained of continuous tremor. Three described emotional problems, and two were under the care of a psychiatrist. Ten were employed full time. Objectively, however, tremor of a pathologic degree was noted in only one of the former workers. This man displayed a fairly coarse tremor that did not abate over the 6 years from start of symptoms. The remaining patients were normal on neurologic and general physical examination.

In summary, the organochlorine insecticides can produce both acute and chronic neurotoxic effects in humans. In most cases acute, exposure produces effects that disappear with time. Unless convulsions develop during intoxication, recovery is usually complete. Convulsions can directly cause neuronal cell loss as can the brain ischemia and anoxia that often accompany any convulsive seizure. In these cases, more severe, persisting damage is possible. Chronic exposures may also produce persisting sequelae. This is particularly true for chlordecone. The true incidence of this response is unknown.

A lack of data makes it impossible to predict accurately the impact of long-term, low-level pesticide exposure on humans. Few animal studies have been undertaken expressly to address this type of risk assessment. Human epidemiologic data suggest that the quality of life may be compromised by the types of exposures experienced by spraymen, but provide no clear picture of the possible impact of lower exposure levels.

Anticholinesterase Insecticides

Organophosphorus esters (OPs) and carbamates are insecticides with a common mode of action. Their capability to inhibit the activity of neural acetylcholinesterase (AChE) makes them effective against pests, but, at the same time, creates toxic potential for humans and other animals. Acute toxicity results from excessive cholinergic stimulation and includes both central and peripheral nervous system components (Table 3-2). Signs include those resulting from stimulation of the muscarinic receptors of the parasympathetic nervous system (increased secretions, bronchoconstriction, diarrhea, bradycardia, urination, miosis); those resulting from stimulation of nicotinic receptors including the ganglia of the sympathetic and parasympathetic nervous systems, as well as the junctions between nerves and muscles (muscle fasciculations, tremors, weakness, or flaccid paralysis); and those resulting from effects in the central nervous system (including convulsions and coma). Chemical structure, lipid solubility, dosage, and pharmacokinetics all contribute to these effects. Treatment with atropine will relieve muscarinic symptoms and significantly and rapidly improve prognosis after exposure to OPs and carbamates. In addition, oximes can be administered to remove OPs from acetylcholinesterase. Appropriate use of oxime antidotes will also relieve signs and improve prognosis in cases of intoxication. These acute effects of OPs and carbamates, and the treatment thereof, are well recognized (Ecobichon, 1982b; Ellenhorn and Barceloux, 1988; Hayes, 1982, pp. 284-462;

TABLE 3-2. Symptoms of Poisoning by Anticholinesterase Insecticides in Humans

Insecticide Class	Signs of Excessive Cholinergic Stimulation	Residual or Repeated Low-Dose Effects
Organophosphorus Esters chlorpyrifos coumaphos dichlorvos diazinon dimethoate disulfoton fenitrothion fenthion malathion parathion ronnel trichlorfon	Muscarinic: Respiratory - dyspnea, increased bronchial secretions, cough, pulmonary edema, cyanosis Gastrointestinal - nausea, vomiting, abdominal cramps, diarrhea Secretory glands - increased sweating salivation, and lacrimation Cardiovascular - bradycardia, hypotension Visual - blurring of vision, miosis Urinary - increased frequency of urination	Central Nervous System: headache, dizziness, incoordination, nervousness, anxiety, irritability, insomnia, confusion, loss of concentration, speech difficulties, depression, impairment of judgment, memory deficits, visual disturbances, ECG changes, fatigue, other psychologic changes Neuromuscular: weakness, temporary paralysis (including muscles of respiration)
Carbamates aldicarb aminocarb benomyl carbaryl carbofuran methomyl propoxur	Nicotinic: Striated muscle - twitching, fasciculations, cramps, weakness, flaccid paralysis Ganglia of autonomic nervous system, sympathetic division - tachycardia, hypertension	Peripheral Nervous System: delayed neuropathy (with specific organophosphorus esters only)
	Central Nervous System: anxiety, restlessness, emotional instability, confusion, ataxia, weakness, coma, convulsions, cyanosis	

Morgan, 1988; Murphy, 1986; Namba et al., 1971) and will not be considered further in the present review.

In addition to the inhibition of neural acetylcholinesterase activity noted above, OPs and carbamates will inhibit activities of cholinesterases in erythrocytes and in plasma. Activities of these enzymes, especially those of erythrocytes, are often used as noninvasive indicators of cholinesterase activities in the central and peripheral nervous systems of humans and other animals.

Residual effects of OPs and carbamates and their relationship to cholinesterase inhibition are also discussed in the paragraphs that follow. In experimental animals given OPs, the activity of erythrocyte cholinesterase has been demonstrated to parallel decreases in the activity of brain cholinesterase, but correlation is not exact and may depend on the specific OP to which the animal was exposed. Although reliability, especially in chronic studies, was suggested to be less than ideal, blood cholinesterase levels are useful indicators of human exposure to OPs, especially in the absence of clinical signs. Blood cholinesterase activity measurements are less significant indicators of carbamate exposure than of OP exposure because carbamates are easily removed from the enzyme, and assay results may be inaccurate (Wills, 1972). Recently, a radioimmunoassay has been described that may resolve some of these problems (Brimijoin et al., 1987).

A neurotoxic effect that will not be discussed in depth as part of the present review is the delayed neuropathy induced by some, but not all, OPs. Signs of this delayed toxicity take time (days to weeks) to appear in humans and in some animal species after exposure. Development of delayed neuropathy is not associated with inhibition of neural acetylcholinesterases as is acute toxicity, but can be correlated instead with an initial phosphorylation and inhibition of another enzyme known as neurotoxic esterase or neuropathic target esterase (NTE) (Johnson, 1982; Lotti, 1986). Clinical manifestations are progressive and irreversible and include weakness, ataxia, and paralysis. This delayed neuropathy initially affects distal portions of axons, primarily of the longest nerve fibers (Davis and Richardson, 1980; Jortner, 1984).

Instead of reviewing the acute toxicity that follows acetylcholinesterase inhibition or the delayed neuropathy that follows NTE inhibition, the present review will explore other neurotoxic manifestations that can be seen in humans exposed to OPs and carbamates, especially neurotoxic effects that have been reported to occur following multiple low-dose exposures, or seem to persist even after recovery from acute intoxication. Although not all studies used these particular compounds, a list of the more familiar OP insecticides would include chlorpyrifos, coumaphos, dichlorvos, demeton, diazinon, dimethoate, disulfoton, fenitrothion, fenthion, malathion, parathion, ronnel, and trichlorfon. The more familiar carbamate insecticides include aldicarb, aminocarb, benomyl, carbaryl, carbofuran, dimetan, methomyl, and propoxur.

Organophosphorus Esters: Residual Effects in Humans Most reports of neurotoxic effects of OPs that appear unrelated to enzyme inhibition have been in humans who have survived an exposure. These residual effects include such behavioral abnormalities as anxiety and irritability, mental confusion, and depression (Ellenhorn and Barceloux, 1988; Hallenbeck and Cunningham-Burns, 1985; Levin and Rodnitzsky, 1976; Savage et al., 1988) (Table 3-2). Other persistent symptoms that have been reported include impairment of judgment, memory deficits, muscle weakness, fatigue, visual disturbances, and electroencephalographic (EEG) changes. Reviews of the literature show that these symptoms have not always been observed, even when specific attempts have been made to assess them (Ecobichon, 1982b; Hayes, 1982, pp.284-435; Karczmar and Ohta, 1981; Levin and Rodnitzsky, 1976; Metcalf and Holmes, 1969; Plestina and Piukovic-Plestina, 1978). In some cases, the detrimental effects that followed OP exposure could be associated with decreases in blood cholinesterase activities; in other cases, enzyme activities were either not inhibited or were not measured. In many cases, individuals with these symptoms improved with time. Some symptoms, however, such as headache, visual disturbances, and EEG changes, persisted for months.

Several recent studies have attempted to correlate persistent neurologic symptoms in humans exposed to OPs with blood cholinesterase activities or with severity of the acute clinical symptoms. In one study of 31 farm workers (Coye et al., 1986), 29 had persistent symptoms, with eye irritation, headache, dizziness, nausea, visual disturbances, weakness, and diarrhea lasting up to 5 to 10 or more weeks after exposure. Blood cholinesterase activities were inhibited, but only to an average of 15.6 percent in plasma and 5.6 percent in erythrocytes. In a later study that included these subjects as well as other farm workers exposed to OPs (Coye et al., 1987), cholinergic signs abated in 4 weeks, but headache, weakness, anorexia, and blurred vision persisted. Blurred vision persisted in some instances throughout the 18 weeks of postexposure observation.

Lasting and delayed toxicities, including death, were also noted when data were collected for a computerized medicinal system that contained 236 records from individuals poisoned with OPs (Hirshburg and Lerman, 1984). At least 4 of 12 individuals given EEG exams had abnormalities, with 2 of these 4 still not normal 3 months after OP exposure. Nine patients complained of depression, confusion, agitation, insomnia, and muscle weakness. Most patients with these persistent abnormalities had been labeled only "mildly poisoned" (symptoms limited to mild muscarinic signs only).

Neuromuscular weakness has been reported to appear in some humans after resolution of the acute OP poisoning crisis by treatment with atropine. This may be a nicotinic symptom of OP intoxication, as the excess acetylcholine can act like a depolarizing, non-competitive neuromuscular blocking agent. Receptor desensitization in the presence of excess acetylcholine may also contribute to the muscle weakness observed (Karczmar and Ohta, 1981). Observers have noted one or more days to pass between exposure and appearance of muscular weakness. Severe, acute cholinergic symptoms (i.e., diarrhea, miosis, sweating, salivation, muscle fasciculations) were no longer present at the time of muscle weakness. One recent report, for example, noted that, in some individuals, weakness was so severe that ventilatory support was required and 3 patients died. The condition of the 7 surviving patients resolved within 18 days (Senanayake and Karalliedde, 1987). Blood cholinesterase activity was not determined in these individuals. The relationship of blood, muscle, and neural cholinesterase activities in humans is unknown. Experiments in animals have demonstrated, however, that OPs can inhibit the muscle enzyme in a dose-related manner that compares to inhibition of the brain enzyme activity (Cohen and Murphy, 1970).

Organophosphorus Esters: Repeated Low-Dose Exposures and Symptoms in Humans. The effects of repeated low-dose exposures of humans to OPs have been noted (Ecobichon, 1982b; Levin and Rodnitzsky, 1976; Metcalf and Holmes, 1969), especially among individuals working with these pesticides. Symptoms reported have included nervousness, memory deficits, fatigue, lassitude, depression, speech difficulties, loss of concentration, reduced cognitive efficiency, and other psychologic changes. Not every investigation has determined that these neurobehavioral symptoms could be associated with exposure to OPs (Bellin and Chow, 1974). Chronic exposure to OPs, for example, was not associated with visual disturbances (Plestina and Piukovic-Plestina, 1978). Some neurologic changes occurred in the absence of blood cholinesterase inhibition (e.g., memory deficit), but in many studies, data on enzyme activities in patients with these symptoms were not available (Ecobichon, 1982b; Levin and Rodnitzsky, 1976; Metcalf and Holmes, 1969).

Two more recent studies examined occupational exposure to OPs in India. One study (Gupta et al., 1984; Kashyap, 1986) included 96 formulation workers exposed to both organophosphorus and organochlorine pesticides. A larger percentage of these workers complained of headache, insomnia, and eye irritation than did the 60 nonexposed controls. The average blood cholinesterase activities for formulation workers were significantly reduced (77 percent of control for plasma, 61 percent of control for erythrocytes). The formulators had also been exposed to chlorinated hydrocarbon pesticides. The second study (Misra et al., 1985) evaluated pesticide workers who had been spraying fenthion regularly for over 8 years. Some of these individuals (<9 percent) complained of persistent aches, parethesias, visual disturbances, and respiratory difficulties. Serum cholinesterase activities averaged 70 percent of control.

Organophosphorus Esters: Animal Studies. Reasons why human beings could experience neurotoxic symptoms as residual effects or as a result of multiple low-dose exposure to OPs can be obtained, in part, from review of data collected in laboratory animals. Animal studies, for example, have indicated that repeated exposures to OPs at dosages that did not elicit clinical signs could cause changes in peripheral nerve excitability and conduction (Anderson and Dunham, 1985; Averbook and Anderson, 1983) and changes in locomotion (Farage-Elawar and Francis, 1987). Behavioral changes and impaired learning have also been noted in rats after administration of OPs, especially with those agents that could reduce activities of blood cholinesterase without effect on brain enzyme activity (Giardini et al., 1982; Karczmar, 1984; Lehotzky, 1982).

Morphologic damage has been noted in some cases in which laboratory animals have been exposed to OPs over long periods of time. Retinal degeneration has been observed in rats exposed to fenthion 100 times in a year (Imai et al., 1983). Liver cholinesterase activity was inhibited in these animals, while cholinesterase activities in blood or brain were not reported. Morphologic changes have also been noted in laboratory animals given an OP (soman) that is not used as a pesticide. This damage has been reported in the absence of clinical signs, although brain cholinesterase activity was 50 percent of normal (Sikora-VanMeter et al., 1987). Exposures producing seizures also caused axonal degeneration and necrosis in brain and muscle (Churchill et al., 1985; Dettbarn, 1984; Sikora-VanMeter et al., 1985), and a one- to three-day reduction in regional brain glucose utilization (Pazdernik et al., 1985). Morphologic changes were associated with behavioral changes in rats surviving soman exposure (Raffaele et al., 1987).

Acute exposure to OPs can also upset the balance of the dopaminergic and cholinergic systems of the central nervous system, a balance which may take some days for recovery (Ho and Hoskins, 1987). OPs may also phosphorylate enzymes of the CNS other than acetylcholinesterase (e.g., monoamine oxidase, ATPase), change levels of cyclic AMP, or alter membrane structure (Marquis, 1986a, 1986b). Any or all of these factors could contribute to neurobehavioral changes that persist after OP exposure.

In addition to the animal studies cited above, which indicate detrimental effects occurred after repeated exposure to OPs, other studies indicate that prolonged exposure to OPs resulted in adaptations that allowed animals better toleration of the cholinesterase inhibition produced by these substances. This tolerance can manifest itself in behavioral adaptations and reduced transmitter release, as well as reductions in the sensitivity and number of muscarinic acetylcholine receptors (Costa et al., 1982; Ho and Hoskins, 1987; Russell and Overstreet, 1987). Memory impairment has, however, recently been demonstrated in OP-tolerant rats (McDonald et al., 1988).

Carbamates: Repeated Low-Dose Exposures and Symptoms in Humans. The literature on repeated low-dose exposure effects of the carbamates is considerably smaller than that on the organophosphorus esters. Residual or multiple low-dose toxicities were not noted in a 1982 review (Ecobichon, 1982a), although the results of a survey published in 1983 (Sterman and Varma, 1983) indicated that as many as 19 percent of individuals exposed to well water containing aldicarb had neurologic complaints, including paresthesias, locomotor difficulties, visual problems, and dizziness. The case report described earlier is a singular example of a well-documented neurotoxicity caused by repeated exposure to a carbamate (Branch and Jacqz, 1986a; 1986b).

Carbamates: Animal Studies. A review of the toxic effects of aldicarb in animals (Risher et al., 1987) revealed that no adverse effects of long-term administration were noted in rats or dogs given oral aldicarb for up to 2 years. Cranmer (1986) cited several studies in which adverse effects of carbaryl were reported (i.e., morphologic changes in the basal ganglia of rabbits, increases in brain protein in rats, and myopathy in swine), but he suggested that carbaryl produced behavioral toxicity only at doses that decreased cholinesterase activities. Tolerance to carbamates has also been reported (Costa et al., 1982).

Two studies suggest that long-term exposure to carbamates can cause neurotoxicity (Desi et al., 1974; Smalley et al., 1969). In the first study, swine given carbaryl orally for 83 days developed histopathologic lesions in muscle and brain that were attributed to a vascular effect of the carbamate (Smalley et al., 1969). Cholinesterase data were not provided. Treated animals developed ataxia, tremors, and paraplegia. In the other, Desi et al. (1974) gave rats a dose of carbaryl that did not inhibit blood cholinesterase activities or cause clinical signs (although cholinesterase was inhibited up to 40 percent in various parts of the brain). After treatment for 2 years, these animals showed decreased maze performance and EEG changes.

Other animal studies have indicated that behavioral changes could accompany cholinesterase inhibition in laboratory animals given carbamates (Ruppert et al., 1983). Like the OPs, carbamates in cholinesterase-inhibiting doses may alter concentrations of monamine neurotransmitters (Cranmer, 1986; Takahaski et al., 1987). These changes, too, could contribute to behavioral effects observed after exposure.

Pyrethrins and Pyrethroid Insecticides

Pyrethrins are natural products derived from the plant *Chrysanthemum cinerariaefolium*. There are six active insecticidal pyrethrins in the commercial product Pyrethrum. Since 1970, a number of synthetic derivatives have been produced that are more stable and more potent than the natural products. In consequence, they have seen increasing use in agriculture and in the home. The synthetic pyrethroids can be separated into two classes (type I pyrethroids such as allethrin, permethrin, and cismethrin and type II pyrethroids such as fenvalerate, deltamethrin, and cypermethrin) on the basis of their signs of poisoning in animals (Cremer, 1983; Gray, 1985; Verschoyle and Barnes, 1972).

Pyrethrum Under practical conditions, Pyrethrum and its derivatives are probably the least toxic to mammals of all insecticides in use (Hayes, 1982). Injury to humans from Pyrethrum has most frequently resulted from the allergenic properties of the material rather than its direct toxicity. Contact dermatitis is by far the most common. Descriptions range from local erythema resembling sunburn, to a vesicular eruption similar to poison ivy (McCord et al., 1921).

Pyrethroids Studies of human populations exposed to the synthetic pyrethroids have not demonstrated important neurologic effects (Fusia et al., 1987; Hayes, 1982; Prinsen and van Sittert, 1980; WHO, 1979). Cyano-containing (type II) pyrethroids have been associated with the production of a unique cutaneous paresthesia. Fenvalerate, for example, produced paresthesia in a high percentage of exposed workers (Knox et al., 1984; Tucker and Flannigan, 1983). Paresthesia developed a number of hours after exposure and was described as stinging or burning which sometimes progressed to numbness. Sunlight, perspiration, and washing exacerbated these symptoms. The effect lasted 12 to 18 hours. Similar responses have been produced in guinea pigs (Cagen et al., 1984; McKillop et al., 1987) and rabbits, and this is being used as an assay for this effect.

Type I pyrethroids produce tremor in mice and rats that is first notable in the limbs and gradually spreads to involve the entire body. Body temperature increases dramatically during tremor. The effects are similar to those produced by DDT. The type II pyrethroids produce intense salivation followed by the development of a coarse, whole-body tremor or wobble. Subjects develop a unique, continuous writhing (choreoathetosis) of the limbs and trunk. Convulsive seizures are common before death.

All of the pyrethroids affect sodium channels in nerve fibers (Narahashi, 1985; Vijverberg and de Weille, 1985). Type I pyrethroids cause repetitive nerve discharge like DDT and delay sodium channel inactivation. Type II pyrethroids produce an even longer delay in sodium channel inactivation. This leads to a persisting depolarization of the nerve fiber, a reduction in the amplitude of the action potential, and eventual failure of axonal conduction. The relative abilities of these pyrethroids to increase the time that sodium channels remain open have been proposed as the explanation for both types of toxic responses (Narahashi, 1985). Type II pyrethroids also bind to the GABA receptor-chloride channel complex (Eldefrawi et al., 1985; Lawrence et al., 1985). They do not, however, appear to have the same effect on GABA-mediated increases in chloride flux through these same channels (Abalis et al., 1986; Bloomquist et al., 1986; Ogata et al., 1987).

Increased peripheral nerve excitability has been observed after exposure to both type I and type II pyrethroids (Takahashi and LeQuesne, 1982). The period of heightened excitability after an action potential is increased in both extent and duration. The increases in duration observed after exposure to type I and type II pyrethroids parallel their known effects on individual nerve fibers and on individual sodium channels (Narahashi, 1985; Vijverberg and de Weille, 1985). These effects may be associated with reports of neuromuscular dysfunction and "sparse axonal degeneration" (Rose and Dewar, 1983). In these studies, all of the reported neuromuscular and neuronal effects disappeared rapidly after termination of exposure.

Little evaluation of the behavioral effects of pyrethroids has been undertaken. They have been reported to reduce spontaneous motor activity and alter startle responses in rats (Crofton and Reiter, 1984; 1987; Hudson et al., 1986) and to modify operant performance (MacPhail et al., 1981; Peele and Crofton, 1987; Stein et al., 1987). The relevance of these findings to the effects that continuing low-level exposure might produce in humans cannot be assessed at this time.

In summary, Pyrethrum has been employed for a long time, and the newer synthetic pyrethroids have been used intensively for the last 10 years. During that time there has been no indication that acute or chronic exposures are likely to produce significant neurologic concerns in humans. The data base, from both human and animal studies, however, is very sparse. There is evidence from the animal literature that neuronal, neuromotor, and behavioral consequences do occur with some exposures. It is not possible to determine whether the exposures experienced by humans put them at risk for these effects.

Herbicides, Fungicides, and Fumigants

Herbicides The use of herbicides now exceeds that of insecticides and fungicides combined. A wide range of chemical classes are used as herbicides, including thiocarbamates, organophosphates, triazines, and chlorophenoxy-, urea-, arsenical-, and acetanilide-based compounds. Herbicides are generally thought to be of low to moderate toxicity to mammals; their toxicity is considerably lower than that of insecticides. Recent reviews have implied mammalian safety because of the selectivity of herbicides for biochemical mechanisms unique to plants (Marquis, 1986). Considerable evidence is available, however, that some major herbicides are neurotoxic in mammals.

Neurotoxicity produced by phenoxyacid herbicides involves prominent neuromuscular effects. 2,4-Dichlorophenoxyacetic acid (2,4-D), for example, produces neuromuscular rigidity (myotonia) in laboratory rats (Squibb et al., 1983; Mattsson et al., 1986). Myotonia has also been reported following exposure of rats to 2-methyl-4-chlorophenoxyacetic acid (MCPA) (Elo and Ylitalo, 1979); however, Hayes (1982c) observed that while myotonia produced by 2,4-D is common to animals, it is unusual in human poisoning episodes, even though the individuals display marked muscle dysfunction. Singer et al. (1982) reported that workers employed in the manufacture of 2,4-D and 2,4,5-trichloroacetic acid (2,4,5-T) had slowed peripheral nerve conduction velocities. These decrements were positively correlated with length of employment.

In contrast to the phenoxyacid herbicides, considerably less is known about the neurotoxic potential of other classes of herbicides. For example, arsenical-based herbicides such as disodium methylarsonate (DSMA) might be expected to produce peripheral neuropathies, although there is no available supporting evidence. Triazines are widely used herbicides in this country and elsewhere, and yet hardly any data exist to suggest whether or not they are neurotoxic. A related compound, aminotriazole, has antithyroid properties and may be expected to produce developmental effects, but again data on neurotoxicity are lacking. It has been suggested that paraquat and related herbicides might be neurotoxic, based on their structural similarity to the neurotoxicant pyridine methylphenyltetrapyridine (MPTP), which produces Parkinson-like symptoms in humans (Kopin, 1985). There is, however, currently no evidence available to suggest that these compounds produce lesions similar to those found in humans and certain other animal species exposed to MPTP.

Recent data suggest that the thiocarbamate herbicides diallate and triallate might produce neurotoxicity in hens (Fisher and Metcalf, 1983) and possibly in rodents (personal communication, W. F. Sette, Office of Pesticide Programs, U.S. Environmental Protection Agency, 1988). Human data are not available on the neurotoxic potential of these thiocarbamates.

Fungicides Some of the more common fungicides include mercurials and organomercurials, organotins (e.g., triphenyltin), dithiocarbamates (e.g., maneb, zineb), and a variety of other chemical classes.

Many organomercury compounds have been found in foods treated with mercurial fungicides (Lu, 1974). Clinical manifestations of mercury poisoning include sensory disturbances, tremor, and ataxia. In addition, psychiatric symptoms were seen in victims of the 1972 Iraqi poisoning episode, in which thousands ingested grain treated with methylmercury (dicyandiamide) fungicide (Maghazaji, 1974). The toxic effects of methylmercury compounds to the adult and developing nervous system are well established and will not be reviewed here (Chang, 1987; Ecobichon, 1982c).

Although certain organotin compounds (e.g., triethyltin and trimethyltin) are known to be neurotoxic (McMillan and Wenger, 1986), little evidence exists that triphenyltin has neurotoxic potential in animals or in humans. A notable exception is the report by Lehotzky et al. (1982) that rats treated for 6 weeks with the fungicide Tinestan (containing 60 percent triphenyltin acetate) showed decreased exploratory activity in a maze and slower extinction (but not acquisition) of a conditioned avoidance response than controls.

Diphenyl is a fungistat that reportedly precipitated a fatal poisoning episode in a worker in a Finnish paper mill (Hakkinen et al., 1973). Subsequent examination of eight coworkers uncovered a host of electrophysiologic anomalies, many of which were still evident 1 to 2 years later (Seppalainen and Hakkinen, 1975). Additional reports of diphenyl-induced neurotoxicity are lacking.

Hexachlorobenzene was originally introduced for the control of fungi on seed grains, especially wheat. Human exposure to hexachlorobenzene has produced neurologic dysfunction. In the mid-1950s over 4,000 Turkish inhabitants accidentally consumed hexachlorobenzene-treated wheat grain, and approximately 10 percent died. The resulting syndrome, called the "new disease" or "black sore," was characterized by severe skin blistering exacerbated by sunlight and mild mechanical trauma (Hayes, 1982). No neurologic or mental disturbances were reported at the time of examination. Follow-up studies, however, revealed prominent neurologic effects including weakness, paresthesias, and myotonia in survivors 25 years later (Morris et al., 1984; Peters et al., 1982).

Animal studies have shown that, although the acute toxicity of hexachlorobenzene is very low, prominent neurologic signs appear after repeated exposures to relatively small dosages (Hayes, 1982). Prominent chronic toxicity may be related to the very slow rate of elimination of hexachlorobenzene from tissues. Rodents treated repeatedly with hexachlorobenzene become hyperreactive and display weakness or paralysis of the limbs. Convulsions may also occur in young rodents (DeMatteis et al., 1961, as cited in Hayes, 1982).

Hexachlorophene was once applied as a broad-spectrum plant fungicide; today it is used by prescription only as an antimicrobial agent. Toxicity studies in humans and animals have shown that acute or short-term repeated hexachlorophene exposure produced intramyelinic edema of the central nervous system (Towfighi, 1980). Signs of intoxication in rodents included hindlimb weakness, paralysis, and decreased sciatic nerve conduction velocity. Recovery generally occurred in many species when exposure was terminated early. Towfighi (1980) provides a detailed description of hexachlorophene neurotoxicity.

Dithiocarbamates are generally used as fungicides and, to a lesser extent, as herbicides. Data on neurotoxic effects in humans are extremely sparse. However, an intriguing case of acute poisoning by a combination of maneb and zineb (manzidan) is described by Israeli et al. (1983) in which the individual complained of weakness and fatigue, headache and nausea, displayed "behavioral confusion" with unclear speech, and finally lost consciousness. Recovery was reportedly complete. Many dithiocarbamates are known to produce neurotoxic effects in a variety of species. A review of the animal literature on dithiocarbamate neurotoxicity can be found in Miller (1982). Thiram, for example, produced signs of poisoning in laboratory rodents, including ataxia and paralysis of the hindlimbs accompanied by damage to the ventral horn neurons, plus degeneration and demyelination of the sciatic nerve (Lee and Peters, 1976). Repeated exposure of rats and dogs to maneb produced weakness of the hindlimbs, incoordination, and tremor (FAO/WHO, 1965, 1968, as cited in Hayes, 1982). Ethylene *bis*-dithiocarbamates are unique in that they are metabolized to the antithyroid agent, ethylenethiourea, and are therefore likely to have effects on the developing nervous system. The role that the common metabolite, carbon disulfide, plays in the actions of dithiocarbamates is suspect but not defined.

Triazole fungicides are relatively new, being widely used as systematically acting agents. No data are available on their neurotoxic potential in humans, although recent laboratory experiments have shown that triadimefon produced hyperactivity and altered schedule-controlled performance in rats (Allen and MacPhail, 1988; Crofton et al., 1988; Moser, 1987). The changes in behavior produced by acute exposure to triazole fungicides resemble those produced by psychomotor stimulant-like drugs.

Fumigants Fumigants include halogenated hydrocarbons (e.g., methyl bromide, ethylene dibromide, and 1,3-dichloropropene), oxides (e.g., ethylene oxide), aldehydes (e.g., formaldehyde), as well as sulfur- or phosphorous-containing compounds (e.g., carbon disulfide, sulfuryl fluoride) and cyanides (Morgan, 1982). Although several fumigants are known neuroto-xicants, the experimental and clinical literature is characteristically sparse. Important exceptions are methyl bromide and carbon disulfide, whose sensory, motor, cognitive, and pathologic effects have been reviewed by Wood (1981) and Alexeeff and Kilgore (1983), respectively, in animals and in humans.

Anger et al. (1986) recently examined a cohort of fumigators who applied formulations containing methyl bromide and sulfuryl fluoride. These workers reported a significantly higher incidence of muscle aches, fatigue, incoordination, and slurred speech. Fumigators also performed more poorly than controls in a broad battery of behavioral tests. Anger et al. (1986) concluded that low exposures to methyl bromide, and perhaps to sulfuryl fluoride, may produce mild neurotoxic effects. Additional studies are clearly needed.

DEVELOPMENTAL NEUROTOXICITY AFTER PERINATAL PESTICIDE EXPOSURE

The neuroanatomic development of the central nervous system and its functional output reflects a sequence of highly ordered and precisely timed events. The consequences of their disruption may range from subtle to severe, and the onset may be gradual or abrupt. The developing animal also displays the greatest capacity for plasticity, such that the affected systems may rearrange or reorganize themselves (Finger and Stein, 1982). In addition to gross measures of perturbation (e.g., embryonic death, intrauterine growth retardation, and external/skeletal/visceral malformations), the development of the structure and the function of the nervous system needs to be evaluated during the prenatal and postnatal periods.

Neurotoxicity resulting from perinatal pesticide exposure is summarized in Table 3-3. Criteria used to study developmental neurotoxicity after perinatal pesticide exposure include: (1) behavioral, neurochemical, or neuropathologic alterations in the offspring in the absence of maternal toxicity;

TABLE 3-3. Selected Studies Concerning Developmental Neurotoxicity After Perinatal Pesticide Exposure

Pesticide (class-agent)	Species	Maternal Toxicity	Embryo or Fetal Toxicity	Neonatal Toxicity	Postnatal Neurotoxicity
Organochlorines					
Endrin	Hamster	behavioral	mortality	wt	behavioral
Endrin	Rat	none	none	none	behavioral
Chlordecone	Rat	none	none	none	behavioral neurochemical neuroendocrine
Organophosphates					
Methyl-parathion	Rat	none[a] neurochemical	none	none	neurochemical behavioral

TABLE 3-3. Selected Studies Concerning Developmental Neurotoxicity After Perinatal Pesticide Exposure (Cont.)

Pesticide (class-agent)	Species	Maternal Toxicity	Embryo or Fetal Toxicity	Neonatal Toxicity	Postnatal Neurotoxicity
Pyrethroids					
Permethrin	Rat	none	NR	none[a]	behavioral
Fenvalerate	Rat	none	NR	none[a]	behavioral
Herbicides					
2,4-D/2,4,5-T	Rat	none[a]	none	none[a]	behavioral neurochemical
Fumigants					
EDB	Rat	none	NR	none	behavioral

none = normal, not different from controls; none[a] = no toxicity at lower dose; NR = not reported; wt = body weight gain reduction.

(2) developmental alterations in the absence of overt teratology; and (3) developmental alterations in the absence of otherwise overt postnatal toxicity.

Perinatal Pesticide Exposure in Humans

Although few cases have been well documented, perinatal pesticide exposure in humans has resulted in chronic neurotoxicity. Exposure to methylmercury fungicide-contaminated wheat resulted in severe impairment of motor and mental development in infants (Amin-Zaki et al., 1974; Curley et al., 1971). Follow-up neurologic examinations revealed cerebral palsy in children exposed prenatally (Amin-Zaki et al., 1979). Another fungicide, hexachlorobenzene, caused muscular weakness, paresthesia, and convulsions in children exposed perinatally (Gucman et al., 1986). In the case of methylmercury, the symptoms of neurotoxicity were the primary manifestation.

Perinatal Pesticide Exposure in Animal Models

Endrin, a cyclodiene organochlorine, altered the development and function of the central nervous system in hamsters and rats (Gray et al., 1981). Endrin also produced gross morphologic defects in hamsters but not in rats (Chernoff et al., 1979; Kavlock et al., 1981). In the first experiment, hamsters received endrin on gestational days (GD) 5 to 14. Endrin produced behavioral alterations along with maternal, fetal, and neonatal toxicity. In the second experiment, rats were orally dosed from GD 7 to postnatal day (PND) 15. Endrin produced no maternal mortality and did not affect litter size, birth weights, or subsequent growth through 115 days of age. Maturational landmarks (eye opening and vaginal opening) were also unaffected. At 16 and 20 days of age, pups showed a dose-dependent increase in locomotor activity. It should be emphasized that these effects of endrin were dose-dependent. Low doses frequently produced behavioral alterations in neonates without other signs of toxicity in the mothers or pups. Higher doses generally produced toxicity in both.

Chlordecone, another organochlorine insecticide, was administered to rats beginning 60 days before mating and continuing through gestation and the first 12 days of lactation (Squibb and Tilson, 1982a). Exposure had no effect on maternal weight gain, litter size or sex ratio, or birth weights of the offspring. Negative geotaxis was the only behavior affected, and that occurred only in males. Altered responsiveness to the dopaminergic agonist, apomorphine, was also noted in males. Seth et al. (1981) noted an elevation of striatal dopamine receptor binding in males. No alterations were noted in GABA, muscarinic, or benzodiazepine receptor binding. Serotonin turnover was significantly greater in offspring exposed to chlordecone in all brain areas evaluated

(Rosecrans et al., 1982). The hypothalamic β-endorphin levels were reduced (Ali et al., 1982).

Chlordecone administered to rat pups on PND 4 induced fine body tremor, increased auditory startle responsiveness, depressed undifferentiated motor activity, and depressed growth in preweanling rats (Mactutus et al., 1982, 1984). These effects are similar to those seen in the adult (Squibb and Tilson, 1982b). They were no longer detectable at 30 days of age.

Methylparathion, an organophosphorous insecticide, administered to pregnant rats (GD 6-20) reduced acetylcholinesterase (AChE) and increased choline acetyltransferase (CAT) activities in the brains of pups and dams. Only minimal cholinergic toxicity was seen in the dams. The lower doses nontoxic to dams reduced AChE but did not affect CAT activity in the offspring. Prenatal exposure also altered cage emergence, locomotor activity, and operant behavior in the offspring. No morphologic changes were observed in hippocampus or cerebellum (Gupta et al., 1985).

Sylianco-Wu et al. (1988) showed that perinatal exposure to formulated products containing type I (Ambush^R, 25.6% permethrin) and type II (Pydrin^R, 30% fenvalerate) pyrethroid insecticides produced developmental neurotoxicity (Sylianco-Wu et al., 1988). Both products affected the righting reflex, forepaw grasping, forelimb placing, cliff aversion, bar holding, and inverted screen test performance. These effects were obtained at very small fractions of the LD_{50} and were unaccompanied by maternal or embryo toxicity.

Chlorophenoxy herbicides, 2,4-D and 2,4,5-T, were administered in a 1:1 ratio to pregnant rats (GD 6 to 15) (Mohammad and St. Omer, 1986; St. Omer and Mohammad, 1987). Contamination by TCDD was considered negligible (0.0125 ppm). Higher doses produced neonatal mortality and decreased weight gain. All doses of the mixture delayed the ontogeny of righting reflex and negative geotaxis. Other behaviors were altered only at doses that also resulted in maternal or neonatal toxicity. St. Omer and Mohammad (1987) found that the mixture increased concentrations of whole brain norepinephrine, decreased its turnover, and delayed the ontogeny of swimming behavior. These data agree with earlier studies (Crampton and Rogers, 1983; Sjoden and Soderberg, 1972) suggesting that gestational 2,4-D/2,4,5-T exposure results in developmental neurotoxicity at doses which do not produce overt maternal or perinatal toxicity.

Neurotoxicity of the fumigant ethylene dibromide (EDB) was assessed in the F_1 progeny of exposed male rats. Paternal EDB exposure altered swimming performance parameters, open-field activity, and cliff avoidance, but perinatal weight gain, righting reflex, and negative geotaxis were unaffected (Fanini et al., 1984).

Behavioral perturbations of the developing organism are often observed following perinatal exposure to any of the major classes of synthetic pesticides. With the cyclodiene organochlorine compounds and several other pesticides, behavioral abnormalities were noted, with little, if any, evidence of nonspecific toxicity (Table 3-3). These studies also emphasize the importance of experimental design for adequately testing the potential of a pesticide to produce developmental neurotoxicity. Such a protocol should include the following information: the species and strain of the animal; the dating of pregnancy and birth; purity, dosage, route, and timing of the administration of the potential toxicant; appropriate vehicle and nutritional controls; presence of maternal, embryo/fetal, or neonatal toxicity; culling and fostering techniques; and statistical analysis procedures, including sufficient sample size, usually based on the litter as the experimental unit. In addition, the use of a neonatal exposure model or the paternal exposure model, although addressing questions different from *in utero* exposure, precludes the problems of nonspecific maternal and fetal toxicity.

CURRENT REGULATORY STATUS OF NEUROTOXIC CHEMICALS

Considerable attention has only recently been focused on the identification and regulation of neurotoxic chemicals. The level of activity is the result of a growing recognition that the nervous system is a target organ for environmental chemicals and that numerous chemicals have never been adequately evaluated for neurotoxicity. The U.S. Environmental Protection Agency (EPA) and other Federal regulatory agencies are charged with enforcing legislation designed to protect both the American public and the environment against the hazards of chemicals. These legislative mandates differ, however, in the extent to which they can require specific toxicity data for regulatory purposes.

Table 3-4 lists the regulatory agencies and the legislative mandates they are responsible for administering. Table 3-5 compares the extent to which the agencies require testing and submission of health-related data for registration purposes, as well as the reporting of health-related data on existing chemicals. Both tables are taken from Reiter (1987). Regulatory agencies differ considerably in their authority to require premarketing approval, toxicity testing, and regulation of exposure. The Occupational Safety and Health Administration (OSHA), for example, cannot require chemical testing by the manufacturer, whereas all other agencies can. On the other hand, only the Food and Drug Administration (FDA) and EPA have authority to require

TABLE 3-4. Major Regulatory Agencies

Agency	Statute and Sources Covered
Food and Drug Administration (FDA) A unit of Health and Human Services with authority over the regulation of and food additives; cosmetics	Food, Drug, and Cosmetics Act for food and feed additives, color in cosmetics, human and animal drugs, and medical devices
Occupational Safety and Health Adminstration (OSHA) A unit of the Department of Labor that regulates workplace conditions	Occupational Safety and Health Act (OSHA) covers toxic chemicals in the workplace
Environmental Protection Agency (EPA) Independent agency (i.e., not part of a cabinet department); administers a number of diverse laws concerned with human health and the environment	Toxic Substances Control Act (TSCA) requires premanufacture evaluation of all new chemicals (other than foods, food additives, drugs, pesticides, alcohol, tobacco); allows EPA to regulate existing chemical hazards not sufficiently controlled under other laws
	Clear Air Act requires regulation of hazardous air pollutants
	Federal Water Pollution Control Act governs toxic water pollutants

TABLE 3-4. Major Regulatory Agencies (Continued)

Agency	Statute and Sources Covered
	Safe Drinking Water Act covers drinking water contaminants
	Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) covers pesticides
	Resource Conservation and Recovery Act (RCRA) covers hazardous wastes
	Marine Protection Research and Sanctuaries Act covers ocean dumping
Consumer Product Safety Commission (CPSC) Regulates a variety of consumer products including household chemicals and fabric treatments	Federal Hazardous Substances Act covers "toxic" household products
	Consumer Product Safety Act covers dangerous consumer products
	Poison Prevention Packaging Act covers packaging of dangerous children's products
	Lead-Based Paint Poison Prevention Act covers use of lead paint in federally assisted housing

TABLE 3-5. Authorities for Toxicity Testing[1]

Agency	Law	Coverage	Authorities		
			pre-marketing approval	testing by manufacturer	reporting of data
FDA	Food, Drug and Cosmetics Act	drugs, foods	X	X	X
		food additives, cosmetics	X	X	
EPA	Federal Insecticide, Fungicide and Rodenticide Act (FIFRA)	pesticides	X	X	X
	Toxic Substances Control Act (TSCA)	industrial chemicals	(X)[2]	X	X
	Clean Act	air pollutants			

TABLE 3-5. Authorities for Toxicity Testing[1] (continued)

Agency	Law	Coverage	Authorities		
			pre-marketing approval	testing by manufacturer	reporting of data
	Resource Conservation and Recovery Act	industrial waste		X	X
OSHA	Occupational Safety and Health Act	occupational exposure			X
CPSC	Federal Hazardous Substances Act	consumer products			
	Consumer Product Safety Act	consumer products			X

[1] Modified from Fisher (1980).
[2] Can require testing based on available data.

premarketing testing of chemicals destined for commerce. The Toxic Substances Control Act (TSCA) gives EPA the authority to require toxicity data from industry; therefore, this law stresses the development ofmethodologies by the scientific community that can be used to collect data for regulatory decision making. Section 4 of TSCA states, for example, that

> "the health and environmental effects for which standards for the development of test data may be prescribed include ... behavioral disorders ... and any other effects which may present an unreasonable risk of injury to health or the environment."

As part of the effort to regulate chemicals (i.e., existing chemicals) under section 4 of TSCA, the Office of Toxic Substances (OTS) of EPA has published a series of test guidelines for assessing neurotoxicity (Table 3-6). No other regulatory agency has developed similar guidelines for neurotoxicity testing. With the exception of EPA's Office of Pesticide Programs' requirement of the "hen test" in evaluating the delayed neurotoxicity of organophosphorous compounds, no other test for neurotoxicity is presently required. The primary tests are intended both to screen compounds for the potential to produce adverse neurobehavioral effects, and to determine if chemicals having neurotoxic effects following acute exposure are also neurotoxic following repeated low-level exposure (Sette and Levine, 1986).

TABLE 3-6. EPA Office of Toxic Substances (OTS) Health Effects Test Guidelines for Neurotoxicity (1985)

Functional observational battery
Motor activity
Schedule-controlled operant behavior
Neuropathology
Peripheral nerve function (neurophysiology)
Acute delayed neurotoxicity of organophosphorous substances
Subchronic delayed neurotoxicity of organophosphorous substances

Schedule-controlled operant behavior is a measure of learned behavior that is also specified in EPA's guidelines (Table 3-6). The neuropathology guideline describes a tiered approach to evaluating structural damage produced by toxicant exposure, beginning at the gross level of evaluation and proceeding to an evaluation at the electron microscope level. The inclusion of an electrophysiologic measure of peripheral nerve function was prompted by the finding that many industrial chemicals are capable of producing peripheral neuropathies. Likewise, inclusion of the two test guidelines for organophosphate-induced delayed neuropathies (acute and subchronic) was prompted by the fact that certain types of organophosphorous esters produce neuropathic damage to both the central and peripheral nervous systems.

Recently, the OTS testing guidelines were adopted in principle by EPA's Office of Pesticide Programs for use in expanded evaluations of the neurotoxic potential of pesticides. In October 1987, the Office of Pesticide Programs convened a subpanel of neurotoxicology experts to hold an open meeting and advise the Science Advisory Panel set up under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) of the need for augmenting current test requirements for evaluating pesticides. The meeting was also in part aresponse to the recent petition of EPA by a number of professional and public health advocacy groups to require expanded neurotoxicity testing for all pesticidal active and inert ingredients. The FIFRA Science Advisory Panel recommended increased screening of pesticides for neurotoxicity using a functional observational battery, motor activity, and neuropathology, with more specific tests of sensory, motor, and cognitive function to characterize neurotoxic hazards.

Further research is needed to achieve an understanding of the full range of neurotoxic effects associated with pesticide exposures using validated test methods. These methods could by promulgated in the form of additional test guidelines or be required in toxicity testing by reference to methods established in the scientific literature. A blend of basic and applied science is essential to the ability of Federal Agencies to detect, characterize, and regulate neurotoxic chemicals.

ASSESSMENT OF NEUROTOXIC EFFECTS

Approaches To Neurotoxicologic Risk Assessment

EPA has traditionally used a two-step procedure in its approach to risk assessment. In the first step, the data base for a chemical is surveyed to determine the highest dose that does not produce any effect (no observed effect level, or NOEL), or the lowest dose that has been observed to produce an effect (lowest observed effect level, or LOEL). In the second step, the NOEL or LOEL is divided by safety factors to establish "safe" exposure levels. For noncarcinogens, the procedure has been to establish an acceptable daily intake (ADI) for human populations over a lifetime that is assumed to produce little or no risk. Recently, the ADI has been replaced by the more neutral term "reference dose."

Lehman and Fitzhugh (1954) were among the first to suggest safety factors in the calculation of ADIs. They suggested that ADIs for food additives be calculated by dividing the NOEL derived from chronic animal toxicity studies by 100. The 100-fold safety factor was assumed to compensate for inter- and intraspecies variability. EPA, in particular the Office of Drinking Water, has adopted this safety factor approach for drinking water (U.S. Environmental Protection Agency, 1980). Under this approach, safety factors are considered as a series of 10-fold divisions of a NOEL or LOEL. In the first step, the NOEL or LOEL is divided by 10 in an attempt to compensate for variability within a human population in instances where chronic human data are available. A second division of the NOEL or LOEL by 10 is assumed to compensate for interspecies variability when chronic animal data are available, but chronic human data are not. This reflects a 100-fold safety factor (10 for interspecies variability, multiplied by 10 for intraspecies variability). A 1,000-fold safety factor is achieved by further dividing by 10 when only subchronic animal data are available. Finally, a safety factor varying between 1 and 10 may be applied in instances where the LOEL is an adverse effect and a NOEL is not available, in an attempt to convert a LOEL into a NOEL. McMillan (1988) has recently illustrated the application of this model in a neurobehavioral toxicity risk assessment for alkyltins.

The safety factor approach to risk assessment used by EPA has many weaknesses. The basic assumption that a 10-fold factor encompasses the variability across individuals within a species and that another 10-fold factor encompasses the variability from test species to human populations is an empirical question that has not been studied in any detail. Furthermore, the NOEL for a chemical depends on many factors, including the size of the data base on the chemical (more testing is likely to produce a lower NOEL), the spacing of doses, and the number of animals used in the individual studies. In fact, the very concept of NOELs and LOELs bases risk assessment on the lowest part of the dose-response curve, where measurements are likely to be least accurate. A number of alternative, mathematically more sophisticated models for risk assessment have been developed (Klaassen, 1986), although some of these models have been based on assumptions about mechanisms of carcinogenesis that might not be relevant for noncarcinogenic end points, for which a threshold effect is usually assumed. Crump (1984) has proposed the calculation of a "benchmark dose" as an alternative to the NOEL. The benchmark dose is a statistical lower confidence limit for a dose producing a predetermined increase in the rate of toxicity, which takes sample size and the shape of the dose-effect curve into account. More sophisticated models for risk assessment, such as Crump's, need to be explored for their utility in the risk assessment of neurotoxicity.

Fundamental to virtually all models of risk assessment is the concept that effects produced in the animal model for measuring chemical toxicity can be extrapolated to humans (Klaassen, 1986). Historically, the concept of extrapolation can be traced to Darwin, and the validity of the concept provides the basis for many of the scientific advances of experimental pharmacology and toxicology. Although toxicologists generally accept the validity of extrapolation of chemical toxicity data from animals to humans, there is some reluctance to extrapolate behavioral toxicity, because of perceptions that human abilities to speak, read, write, compose music, etc., make them qualitatively different from animals. Such notions were foreign to Darwin (for example, in his *Expression of the Emotions in Animals and Man*, published in 1872) and have not been supported by the experience of the pharmaceutical industry, which has successfully screened drugs for behavioral activity on the basis of animal tests for more than three decades. Although neurotoxicity data on environmental chemicals are less extensive, in general, agents shown to produce neurotoxicity in humans are active in animal models, as documented earlier in this chapter.

Many neurotoxicants that produce clear neuropathologic effects in one mammalian species have shown similar neurotoxicity in other species. In those instances where human exposure has been well documented, extrapolation from animals to humans is usually validated. For example, methylmercury, lead, and triethyltin produce neurotoxic effects in animals qualitatively similar to those they produce in man (Chang, 1987). In those instances where species-specific lesions occur, pharmacokinetic differences are frequently involved (Evans and Daniel, 1987). For example, both methanol (Andrews and Snyder, 1986) and MPTP (Kopin, 1985) produce characteristic lesions in humans that are not seen in rats due to different metabolic pathways in the rat for these chemicals; however, this does not mean that appropriate neurobehavioral tests in these animals would not detect some functional changes produced by these chemicals upon which a NOEL could be based for regulatory purposes. Most chemicals that produce behavioral changes also show good extrapolation from animals to humans, as shown by the success of animal models in predicting the clinical activity of a variety of psychoactive drugs in humans (Glick, 1976). It does not appear that there is any simple phylogenetic relationship between species sensitivity and the effects of chemicals. For example, the order of species sensitivity to the effects of trimethyltin on schedule-controlled behavior is pigeon > monkey > mouse > rat (McMillan, 1988), the order of sensitivity to triethyltin is pigeon > rat > mouse (McMillan and Wenger, 1985), and the order of sensitivity to methylmercury is monkey > mouse > pigeon > rat (Evans and Daniel, 1987).

If the fundamental assumption of relevance between animal and human neurobehavioral toxicity of pesticides is accepted, it remains necessary to quantify the relationship for purposes of risk assessment. The mechanism for doing so is the comparison of dose-response relationships across species (Klaassen, 1986). Unfortunately, very few systematic species comparisons have been developed for any type of toxicity, much less neurobehavioral toxicity, so extrapolation is usually done from data collected only in rats or mice.

Another difficulty in quantitative extrapolation has already been mentioned. In developing risk-assessment models using NOELs or LOELs, we are working at the lower end of the dose-effect curve where accuracy is suspect. If a particular pesticide-induced neurotoxicity occurs infrequently in the population at low doses, then very large numbers of animals would be required to establish the effect. Because it would be prohibitively expensive and time-consuming to collect neurotoxicity data for a pesticide in a large population of animals for which the baseline neurotoxicity in untreated animals has not been established, we are forced to explore pesticide toxicity with high exposure levels in smaller groups of animals. Thus, risk assessment has the dual problem of extrapolation from high to low doses, as well as across species. A few unfortunate accidental exposures of human populations have provided the opportunity to compare high-dose effects with data from epidemiology studies on the effects of repeated low-dose exposures and with other data from experimental animals; but for the great majority of pesticides of concern, a data base for neurotoxicity risk assessment is lacking (NRC, 1982).

A related problem concerns the extrapolation of data derived from acute pesticide exposure to situations involving chronic exposure. Few studies are available in the literature on the consequences of lifetime exposure to any neurotoxicant. In fact, the neurotoxicity data base from animal studies is largely based on acute exposure, or repeated exposure for a relatively small portion of the animals' life span. Repeated exposure to a neurotoxicant can result in cumulative effects, tolerance, or effects similar to those observed with single exposure. The effects of chronic administration of acrylamide on grip strength and locomotor activity (Tilson et al., 1979) represent an example of cumulative effects, whereas the tolerance that develops to organophosphate pesticides during chronic exposure is well recognized (Murphy et al., 1982). Furthermore, discontinuation of chronic exposure to a neurotoxicant can produce the functional disruption characteristic of drug withdrawal in a drug-dependent animal or can result in partial to full recovery of function. Complete recovery of some functions can occur despite gross neuropathology, due to the redundancy and plasticity of the nervous system (McMillan and Wenger, 1985).

Despite clear evidence, documented earlier in this chapter, that both acute and chronic exposure to pesticides can result in long-lasting neurotoxicity, no systematic attempts have been made to establish LOELs and NOELs for the neurotoxic effects of pesticides. EPA has not required pesticide manufacturers to provide the necessary neurotoxicity data base for use in risk assessment through its regulatory authority, with the exception of the delayed neuropathy test in hens for organophosphate neurotoxicity. The failure to develop the needed data base for neurotoxicity risk assessment has not been due to a lack of appropriate test methods, since numerous select committees have been in agreement in recommending both neuropathologic observations and functional behavioral measurements for more than a decade (National Academy of Sciences, 1984; McMillan, 1987). The widespread exposure of human populations to pesticides and the serious consequences of neurotoxicity to those exposed and to society demand that neurotoxicity testing become a routine requirement for establishing LOELs and NOELs under the current EPA approaches to risk assessment. Obviously, risk assessment performed on the basis of neurotoxicity testing will be subject to the same limitations imposed by current risk assessment models on the interpretation of all other types of toxicity testing. Increased emphasis should be given to research on behavioral, psychologic, motor, sensory, autonomic, and cognitive processes that will test the assumptions underlying current risk assessment practices and thus lead to the development of improved risk assessment models.

Structure-Activity Relationships

In conjunction with the empirical approach to neurotoxicity risk assessment outlined above, it would be desirable to be able to predict possible neurotoxicity from an examination of structure-activity relationships (SARs), comparing the structures of pesticide candidates with those pesticides already in use that have an adequate toxicity data base. In some instances, such predictions are possible while, in others, associations are either difficult or impossible to make.

The vast array of chronic symptoms reported in studies and anecdotes of humans exposed acutely or chronically to organochlorine, organophosphorus ester, and carbamate insecticides has defied study in animal models primarily because of the subjective nature of the signs and symptoms (see sections on organochlorine and anticholinesterase insecticides). The animal models being developed address only those aspects of neurotoxicity that can be measured with existing behavioral methodology.

Distinct differences can be seen between the dichlorodiphenylethane and cyclodiene classes of organochlorine insecticides. The action of the former appears to be largely associated with the peripheral nervous system, whereas the target of the latter class appears to be localized in the CNS (see section on organochlorine insecticides). At the cellular level, several mechanisms appear to be involved in altering nerve function, including interaction with the sodium channel, the GABA receptor, the Na^+/K^+ pump, calcium binding, and membrane stability. While the dichlorodiphenylethane and cyclodiene classes of chemicals share some common mechanisms, there are distinctive differences. Within limits, the actions of organochlorine insecticides are predictable on the basis of structure.

Certain organophosphorus esters (phosphates, phosphonates, and phosphinates) have been associated with well-defined organophosphate-induced delayed neuropathy (OPIDN) in humans and in certain animal species (Ecobichon, 1982b; Johnson, 1982). A test was developed using the adult hen to assess the neurotoxic potential of every pesticide (U.S. EPA, 1985). However, only those esters (phosphates, phosphonates, phosphinates) causing a significant inhibition of the neuropathic target esterase (NTE) will elicit the characteristic OPIDN. Thus, the standard hen test does not adequately detect forms of neurotoxicity other than OPIDN. There are additional drawbacks to this test method. First, with some phosphate esters, acute cholinergic toxicity is produced in the hens prior to sufficient inhibition of NTE. In some cases, this difficulty can be circumvented by prophylactically protecting the hens from acute toxicity, thus allowing a higher body burden of the toxic agent to be attained. Second, organophosphorus esters that undergo biotransformation *in vivo* to neurotoxic metabolites (i.e., phosphorothioates desulfurated to form phosphates) may do so at too slow a rate to attain high enough levels to elicit sufficient NTE inhibition. In certain instances, this difficulty may be overcome by administering the agent dermally rather than orally. Third, interspecies metabolic differences may preclude the biotransformation of the agent into the reactive form essential for sufficient inhibition of NTE.

In contrast to organophosphorus esters, SARs for the pyrethroid ester insecticides appear to be more distinctively related to chemical structure (see *Pyrethrins and Pyrethroid Insecticides*). The cutaneous paresthesia is highly specific and occurs only with the type II pyrethroids containing a cyano-substituent. These produce tingling and numbness when applied to the skin of humans and signs of similar sensory changes in rats, guinea pigs, and rabbits. The other type I pyrethroids do not elicit this distinctive effect, even at high concentrations (Kolmodin-Hedman et al., 1982; LeQuesne et al., 1984; Tucker and Flannigan, 1983). When administered orally or intraperitoneally to rodents, the type II pyrethroids elicit distinct signs and symptoms of toxicity not observed when type I pyrethroids are given.

Structure-activity relationships for herbicides, fungicides, and fumigants have not been described.

Mixtures

The difficulties encountered in assessing the hazard to human health following exposure to mixtures of pesticides must be addressed because of the dearth of information available in the published literature. Problems can arise from:

1. The purity of the agent(s) being studied, with considerable data being generated in studies using analytical grade (>99%) chemical, whereas the technical-grade agent used commercially may contain more than 10 percent impurities or isomers of the active ingredient, and thereby possess a different spectrum of biologic activity. *Example*: The isomalathion constituent of technical-grade malathion is responsible for greater toxicity than is normally seen with this organophosphorus ester (Aldridge et al., 1979).

2. Changes, with time, in the composition of the technical-grade pesticide during storage under less than ideal conditions, with the formation of more toxic isomers, new products, etc. *Example*: Fenitrothion converts into the isomer *s*-methylfenitrothion, which is approximately 1,000 times more toxic than fenitrothion (Myatt et al., 1975).

3. The purposeful introduction of an agent into a formulation with the intention of enhancing the biologic activity of the pesticide. *Example*: The addition of the synergist piperonyl butoxide to pyrethroid preparations blocks the *in vivo* biotransformation of the insecticide.

4. The accidental or purposeful mixing of two different pesticides in the same tank before spraying, with potential incompatibility and solubility problems or synergistic effects.

5. The presence of so-called "inert" ingredients (cosolvents, suspending agents, and emulsifiers) that may, in addition to eliciting their own

toxicity, enhance the uptake of the pesticide. Particularly hazardous are concentrates, not only because they contain higher levels of active ingredients but also emulsifiers and solvents to enhance absorption. *Example*: Paraquat, when ingested as a concentrate, is almost always lethal, whereas fatalities are much less frequent when diluted preparations are ingested. The mechanism is believed to involve emulsifier or cosolvent enhancement of absorption of the active ingredient to such an extent that the normal defensive mechanisms, such as biotransformation, are unable to cope with the amount of agent (Haley, 1979; Smith, 1987).

6. Exposure to multiple chemicals of the same pesticide class or to those of different classes, usually resulting in an alteration of the biotransformation of one or more agents and thereby increasing the toxicity of at least one agent. *Example*: Malathion toxicity is increased by prior administration or coadministration of other organophosphorus esters (parathion, EPH, tri-orthocresyl phosphate) that effectively inhibit the tissue carboxylesterases essential for the detoxification of malathion (Murphy, 1969).

The above examples provide compelling reasons why toxicologic studies should be conducted using commercially available products or even end-use formulations. Few studies have assessed the toxicity of end-use, dilute formulations in animals (Breckenridge et al., 1982; 1986) or the ability of emulsifiers and cosolvents to alter the toxicity of active ingredients. The complex problems of tank mixes, exposure, and adverse health effects in occupationally exposed individuals have not been addressed, even though such incidents are known to occur.

Susceptible Populations

In addition to known differences in susceptibility to pesticide toxicity related to age, sex, health status, etc., evidence suggests that genetic composition also affects susceptibility. The terms "pharmacogenetics," ethnopharmacology," and, more recently, "ethnotoxicology" have evolved to describe studies in which pharamacologic and toxicologic differences in responses to drugs and other chemicals are investigated in individuals demonstrating genetic polymorphism or different ethnic origins. Unfortunately, such studies have not been extended to pesticides in any consistent effort to explore possible genetic and ethnic differences.

One phenomenon long known is the fact that black males, even when adjusted for age and socioeconomic status, have higher serum levels of total DDT (DDT and DDE) than do white males (Davies, 1972; Davies et al., 1972). This peculiar situation holds only for DDT, not for other organochlorine insecticides (dieldrin, ß-BHC, lindane, and heptachlor epoxide), suggesting a difference in the storage, metabolism, and/or elimination of this agent (Kutz et al., 1977). More recent studies have shown that the temporal decline in body burden of total DDT over the past decade, as measured by adipose tissue levels, has been significantly slower in blacks (Davies, 1987). The results point to distinct but unknown ethnic differences in either mobilization from storage depots or biotransformation of DDT, or both. Whether or not this phenomenon is the consequence of a genetic difference remains to be studied.

There is no evidence of any populations being particularly susceptible to anticholinesterase-type insecticides, despite the fact that there are known genetic differences in serum pseudocholinesterase and arylesterase (Kalow, 1962; Carro-Ciampi et al., 1984). The genetic polymorphism of the pseudocholinesterase does not extend to the nervous tissue acetylcholinesterase, the target of both organophosphorus esters and carbamates. The serum arylesterase (aromatic or A-esterase, EC 3.1.1.2), the enzyme occasionally called "paraoxonase," preferentially hydrolyzes aryl (phenol, naphthol, indole) esters of short-chain aliphatic or phosphoric acids (Ecobichon, 1982b). The rates of paraoxon hydrolysis by the atypical and normal enzymes are markedly different (Carro-Ciampi et al., 1981, 1983). It is not known whether this enzyme plays any significant role in the overall degradation of an organophosphate in a poisoning situation. The ubiquitous carboxylesterases (carboxylic acid ester hydrolases, EC 3.1.1.1), found in tissues of all living organisms, are capable of hydrolyzing a wide spectrum of aliphatic and aromatic esters of short-chain fatty acids and phosphoric acid (Ecobichon, 1982b). Genetic anomalies of this class of enzymes in humans have not been reported in the literature to date, but differences do occur in insects and mammals, leading to tolerance toward insecticides as is indicated below. Genetic polymorphism is a factor to be considered when assessing the exposure of various ethnic populations to pesticides.

The development of pesticide resistance is an extremely widespread phenomenon in insects, the classic example being the malathion-induced selection of strains of resistant mosquitos (*Culex tarsalis*) that had high levels of "malathionase" activity, this enzyme being a carboxylesterase (Matsumura and Brown, 1961). Relatively few vertebrate species have developed pesticide resistance. Susceptible and resistant strains of pine voles and pine mice have exhibited 7- to 12-fold differences in toxicity to endrin (Webb and Horsfall, 1967; Hayes et al., 1975). With organophosphorus esters and carbamates, there are suggestions of potential tolerance to some agents based upon enhanced biotransformation by nonspecific carboxylesterases (Ecobichon, 1982a,b). Carboxylesterases play little role in the detoxification of other insecticides since most of the organophosphorus esters and carbamates possess aryl substituents (Ecobichon, 1982a,b). However, genetic polymorphism of plasma and tissue carboxylesterases has been reported in certain strains of rabbits, marked differences in pharmacologic and toxicologic responses being observed following treatment with such drugs as atropine and procaine (Ecobichon and Comeau, 1974). As with humans, the possibility of genetic polymorphism should be considered when using strains and species of animals to assess neurotoxicity of pesticides.

FUTURE DIRECTIONS

In the past, in the United States, neurotoxicologic and neurobehavioral sequelae caused by pesticide exposures have been recognized more by happenstance than by design. From a clinical point of view, neurologic deficits due to pesticide exposure have been recognized through (1) the occurrence of clusters of neurologic symptoms, (2) case reports and case series, and (3) epidemiologic studies. From an experimental point of view, much of the neurotoxicity data from animals have been gleaned from standard toxicity testing protocols. This leaves great gaps in our knowledge of the neurotoxic potential of pesticides.

Identification of Clusters of Neurologic Deficits

Clusters of pesticide-related neurologic deficits have been identified in several studies of the occupationally exposed worker. A good example was the occurrence of paralysis in workers employed in the manufacture of leptophos in a Velsicol plant in Texas (Xintaras et al., 1978). At least nine workers were seriously affected and the simultaneous involvement of both the peripheral and central nervous systems were initially thought to be due to multiple sclerosis. It was not until later, when the cases were more systematically studied, that the neurologic syndrome was traced to leptophos exposure. Cholinesterase activities in these workers were normal. Concerns about the safety of leptophos had previously been raised by Kilgore (1972) during his investigation of hindleg paralysis in 1,300 water buffalo in Egypt (Shea, 1974). This incident led to the inclusion of the hen test in requirements for the registration of pesticides.

Case Studies

Sporadic pesticide-related neurologic and neurobehavioral deficits have been observed in hospital emergency rooms. A recent example was the report of an unexpected flaccid paralysis which developed 24 to 96 hours after OP insecticide poisoning (Senanayake and Karalliede, 1987). The paralysis involved proximal limb muscles and the muscles of the neck and was unresponsive to atropine and oxime therapy. Four of 10 patients died of respiratory failure. The investigators called this an intermediate syndrome because of the relatively delayed appearance of signs and symptoms. Examples such as this emphasize the important role that the astute and observant physician can play in recognizing neurologic sequelae associated with both acute or chronic pesticide exposure. Such examples indicate the need for a systematic monitoring program of human pesticide poisonings if the true incidence and severity is to be thoroughly documented.

Epidemiologic Studies

Few epidemiologic studies have related chronic low-level pesticide exposure to neurobehavioral toxicity. Savage et al. (1988) performed extensive neurologic and psychologic testing of 100 persons who had previously experienced acute OP insecticide poisoning. This population was compared to controls matched by race, sex, and educational and occupational class. None of the controls admitted to any occupational exposure to pesticides, whereas 96 percent of the poisoned cohorts had occupational exposure. The pesticide-exposed group had experienced their acute poisoning some 9 years prior to the study and 78 percent had been hospitalized. Although no statistical differences were observed in the neurologic functions between the two groups, significant differences were observed in a number of psychologic tests, with the control group scoring higher. Generalizations from such studies are difficult because there are several potential sources of bias, including selection of subjects on a demographic rather than on a psychologic basis. Many sources of bias can be overcome through prospective studies.

Anger et al. (1986) prospectively evaluated the neurobehavioral toxicity in California workers exposed to methyl bromide and/or sulfuryl fluoride for over 1 year. Controls were unexposed individuals from the same industry. More symptoms were reported in the methyl bromide fumigators than in controls. In addition, this group was less efficient in performing 23 of 27 behavioral tests. The group exposed to sulfuryl fluoride also reported more symptoms and exhibited reduced performance in all of the cognitive tests. Selection bias, however, resulted in interpretative difficulties due to differences in age, race, socioeconomic background, education, job types, geographic location, and alcohol use. Despite the demographic and physiologic differences, it was possible to recognize subtle, work-associated, neurobehavioral changes (Anger, 1984).

Factors affecting neuroepidemiologic appraisal of pesticide workers include the following: (1) the extent to which exposure can be quantified; (2) the multiplicity of chemical exposures; (3) the sensitivity and specificity of the neurobehavioral tests; and (4) the time taken to conduct the tests. Baker et al. (1985) suggested that selection bias and demographic variables would present interpretative problems in most cross-sectional studies of behavioral differences in workers exposed to pesticides. They also emphasized the superiority of prospective studies over case-control studies. The development of a computer-assisted battery of tests should simplify the implementation of prospective studies of pesticide-exposed workers (Baker and Letz, 1986).

Data Gaps in Epidemiologic Studies

Pesticide management is changing so rapidly and is so vastly different in the developed and developing world that many data gaps exist in information about the agent, the host, environmental circumstances, and especially neurotoxic sequelae of pesticide poisonings. The OP insecticides are the chemical class of greatest public health concern. Although much is known about neurotoxic aspects of acute pesticide poisoning, there is little information available on the subtle and chronic effects of such OP insecticide exposures. The same is true for other groups of pesticides; and there is a great need to ascertain whether peripheral and central neurotoxic effects, along with behavioral problems, are occurring in association with worker exposures to numerous other classes of insecticides, fumigants, fungicides, and herbicides.

Apart from the paucity of information on neurotoxic complications, the most important data gap is the absence of reliable data on the number of pesticide poisonings that are occurring throughout the world. It is somewhat incongruous that, after all these years, information is not readily available, even in the United States, on the incidence and severity of pesticide poisonings (Davies, 1987). An equally important data gap is the absence of a NOEL or a LOEL for pesticides that is derived from sensitive tests of neurobehavioral dysfunction in animals.

Data Gaps in Animal Studies

Most experiments in the literature on the neurobehavioral effects of pesticides in animals have focused on acute effects. Due to the short-term reversible nature of these effects, such studies have frequently established threshold levels of exposure, even though they were not necessarily undertaken with this goal in mind. Considerably less data are available on neurobehaviorally determined threshold doses following repeated long-term exposures. Clearly, more data are required on the effects of long-term exposures to pesticides. These studies should focus on how pesticide effects may change as a function of dose, dose rate, and duration of exposure, as well as on postexposure recovery. Pesticide and metabolite concentrations in target tissues should be included to distinguish effects due to a change in sensitivity of the nervous system to repeated exposure from those effects due to accumulation of the pesticide in the nervous system.

Although data are available in regulatory office files on threshold levels for non-neurobehavioral end points associated with repeated pesticide exposures, these data do not usually provide information on the threshold levels for neurobehavioral deficits because the tests used to collect the data were nonspecific. Subdivision F of FIFRA states that careful cage-side observations are required to detect effects suggestive of a pesticide's action on the nervous system. There are, however, no details regarding the types of effects to be noted (other than those associated with cholinesterase inhibition) or the ways in which tests should be conducted. The planned acceptance by the EPA's Office of Pesticide Programs of the neurobehavioral test guidelines (see Table 3-6) will represent a significant step forward in reducing the uncertainties with which neurobehavioral evaluations are used in testing for the effects of pesticides (Burin, 1987).

Prospective Studies

It is essential that the neurologic and neurobehavioral potential of low-level and prolonged exposures to pesticides and pesticide mixtures be ascertained since the probability of subtle adverse health effects is very real. For most pesticides, the immediate risks of acute exposure are well understood, but there is little information on the effect of chronic exposures on the delayed sequelae of acute exposure and even less on long-term, low-level exposure.

The World Health Organization and the United Nations Development Program are sponsoring a 10-nation prospective collaborative study of the neurotoxic and behavioral effects of OP insecticide exposures in workers (Maroni et al., 1986). Two developments that should facilitate such an ambitious study are improved exposure indices using biologic markers and computer-assisted behavioral and neurologic evaluations. Developments such as these enhance the testing of large cohorts under standardized conditions.

With present pesticide use patterns, the potential for adverse neurotoxic health effects exists in three groups: (1) those acutely poisoned; (2) those chronically exposed in the workplace; and (3) those incidentally exposed in the general population. Additional pertinent data could be obtained through follow-up studies on acutely poisoned individuals. Those occupationally exposed constitute a valuable population for the study of chronic neurologic and neurobehavioral deficits. Exposure of the general population stems largely from trace amounts of pesticides in air, food, and water. Neurologic and neurobehavioral deficits are less likely to be detected in this population in the absence of effects detected in the occupationally exposed population.

Monitoring and surveillance of workers must be expanded because of the large number of people involved in the pesticide industry who are at high risk. The National Institute of Occupational Safety and Health (NIOSH) has developed a Sentinel Event Notification Systems for Occupations Risks (SENSOR). NIOSH suggests that surveillance of exposed workers should go beyond the mere collection of data and include active preventive programs. The SENSOR program offers assistance to states for selected occupational conditions, including pesticide poisoning. The purpose of the program is to assist state health departments in developing sentinel, physician-based reporting of occupational problems and in conducting follow-up and intervention.

EPA's NHANES III program is designed in part to obtain health-related information from the general public via the monitoring of 30,000 Americans. Unfortunately, NHANES III is not specifically designed to address pesticide-related neurotoxicity.

RECOMMENDATIONS

1. We recommend the development of an adequate surveillance and reporting system, including data on incidence and severity of acute, episodic, and chronic exposure to pesticides, to establish a comprehensive data base on human exposure and the consequences thereof.

2. We recommend that improvements in epidemiologic assessment be addressed, including the addition of neurologic and neurobehavioral end points and the expansion of such investigations to the identification of additional populations at risk.

3. Presently, there is an inadequate data base for neurologic and neurobehavioral effects of pesticides in animals. It is imperative that such a data base be established.

4. We recommend that this neurologic and neurobehavioral data base be developed using the best currently available techniques as exemplified by those guidelines promulgated by EPA (1985).

5. We recommend that such test protocols be required for the registration and reregistration of all pesticides and formulation ingredients.

6. We recommend that this information serve as the basis for the quantitative risk assessment process for the protection of human health.

7. We recommend that research be conducted to explore new methodology to detect and quantitate neurologic and neurobehavioral deficits both in animals and humans in order to improve the risk assessment process.

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CHAPTER 4

CARCINOGENIC EFFECTS OF PESTICIDES

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INTRODUCTION

Pesticides are, by design, biologically active chemicals. Potential carcinogenic effects were almost immediately of considerable interest as evidenced by the large number of pesticides tested in bioassays as early as the 1960s (Hayes, 1975; Innes et al., 1969). Experimental tests continue to provide most of the information regarding carcinogenicity of pesticides, which accounts for the heavy reliance by regulatory and licensing agencies on animal data for overall evaluation of cancer risks. Development of epidemiologic approaches to evaluate cancer risks of pesticides has lagged behind experimental investigations with few studies attempted until the 1970s. The epidemiologic effort, however, is now growing rapidly and supplements laboratory investigations. The International Agency for Research on Cancer (IARC) lists over 40 pesticide-related epidemiologic studies underway in several countries around the world (Coleman and Wahrendorf, 1989). Occupational groups exposed to pesticides are being evaluated; these include farmers, forestry workers, grain handlers, horticulturists, manufacturers, applicators, and veterans. Pesticides have also been evaluated in case-control studies of cancers of the lymphatic and hematopoietic systems, nasal cavities, nasopharynx, kidney, liver, colon, brain, and connective tissue. The quality of epidemiologic studies is improving, but exposure assessment remains a problem.

Analyses of the time pattern of cancers in the USA do not indicate that increases in manufacture and use of pesticides during the last few decades have resulted in epidemic increases in cancer in the general population (Pickle et al., 1987). Increasing mortality rates for lymphatic and hematopoietic tumors in agricultural areas in the central states (Pickle et al., 1987), however, are intriguing given the reported associations between these tumors and some pesticides. A major review (Doll and Peto, 1981) concluded that the majority of cancers were attributable to non-occupational factors, such as cigarette smoking and diet, and that occupational and environmental exposures (including pesticides) accounted for less than 10% of cancer cases, although the validity of this estimate has been debated.

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Nevertheless, there are two reasons why pesticide exposure has received, and should continue to receive, considerable attention from cancer researchers. First, although the risks to the general population appear to be small, there may be significant risk to workers receiving heavy occupational exposure to pesticides. Second, as with other potential occupational carcinogens, pesticides are of particular interest because the exposure is often readily controllable.

Most pesticides, particularly those requiring a crop tolerance, have been tested for carcinogenicity, although not with protocols of equal quality. The objective of this selective review was not to evaluate evidence regarding the carcinogenicity of all pesticides. The focus was on agricultural chemicals with both epidemiologic and experimental data available from the National Cancer Institute/National Toxicology Program (NCI/NTP) and EPA to gain insight into the comparability of the approaches. Stage of action, mechanisms of action, and exposure differences have received special attention in an attempt to reconcile differences and to identify promising areas of research.

THE CARCINOGENIC POTENTIAL OF PESTICIDES BASED ON EPIDEMIOLOGIC STUDIES

In contrast to experimental studies where the effects of specific pesticides can be evaluated, epidemiologic studies are difficult to design and interpret because humans are rarely exposed to only one pesticide. This problem of how to account for exposure to more than one pesticide has stifled epidemiologic research on pesticides for years. Although this difficulty should not be minimized, it is a limitation that applies to all epidemiologic studies, whether the exposure of interest is dietary or occupational and whether it is tobacco smoke, alcohol, or a virus. Humans are repeatedly exposed to a variety--usually an unknown number--of potentially hazardous agents. This has not, however, prevented epidemiologic studies from establishing the carcinogenic nature of a number of environmental agents including tobacco smoke, alcohol, asbestos, arsenic, and benzene (IARC, 1987).

Evaluation of cancer risks for specific agents in the face of exposure to multiple factors is possible. Persons who handle pesticides as part of their jobs typically come into contact with a particular combination of pesticides.

Grain handlers have used fumigants such as ethylene dichloride, ethylene dibromide, phostoxin, and malathion. Right-of-way applicators use herbicides, but not insecticides. Farmers, as a group, use nearly every pesticide developed, but those used by the individual farmer depend upon the type of farming operation. Wheat farmers in the United States use more herbicides--2,4-D accounts for 70% of the total--than insecticides (Eichers et al., 1978). 2,4-D also predominates in southern Europe and the Soviet Union, whereas (4-chloro-2-methylphenoxy) acetic acid is more common in central and northern Europe. On the other hand, corn farmers are much heavier users of insecticides and use less 2,4-D. The challenge for epidemiologists is to design studies that take advantage of these variations in exposure patterns to evaluate the cancer risks of specific pesticides or classes of pesticides, to assess the effect combined exposure may have on carcinogenic potential, and to evaluate carcinogenic risks by dose.

Ecologic and General Occupational Studies

Early epidemiologic studies designed to uncover associations between general pesticide use and cancer risk used readily available data to evaluate risks among persons in occupations where contact with pesticides was likely (Fasal et al., 1968; Milham, 1971). For example, general occupational studies in a number of countries noted elevated risks for cancer among farmers (Blair et al., 1985). Geographic correlational studies of cancer patterns and pesticide use conducted in several countries have also suggested that risks of certain cancers are elevated in rural areas where pesticides are widely used in agriculture, although the role of zoonotic viruses, fuels, and dusts could not be definitely excluded in these studies. Tumors apparently associated with pesticides in these early studies include cancers of the lymphatic and hematopoietic system, brain, prostate, stomach, skin, and connective tissue (Blair et al., 1985). Although these investigations were incapable of evaluating the role of specific agricultural exposures, in subsequent death certificate studies, investigators attempted to relate cancer risks to the production of specific agricultural commodities to sharpen hypotheses (Blair and Thomas, 1979; Blair and White, 1981; Blair and White, 1985a; Burmeister et al., 1983; Burmeister et al., 1983a; Cantor, 1982; Cantor and Blair, 1984). Given the potential for exposure misclassification in such an approach, it is not surprising that this was only moderately successful. Associations occurred, however, between lymphatic and hematopoietic cancer and corn production and dairy farming, agricultural activities associated with heavy pesticide use (Blair and Thomas, 1979; Burmeister et al., 1983a). Despite limitations, these early studies indicated that occupational exposure to pesticides may be associated with development of certain cancers.

Studies of Occupational Cohorts Exposed to Insecticides

Cohort studies to evaluate groups with pesticide exposure have included manufacturers of organochlorine compounds (Ditraglia et al., 1981; Ribbens, 1985; Shindell and Ulrich, 1986; Wang and MacMahon, 1979a; Wong et al., 1984), applicators treating termites and other pests (MacMahon et al., 1988; Blair et al., 1983; Wang and MacMahon, 1979), and agricultural applicators (Barthel, 1981; Morgan et al., 1980). Although specific exposures experienced by individual subjects could not be documented, exposure in several groups was limited predominantly to some of the commonly used organochlorines such as chlordane, heptachlor, DDT, dieldrin, and aldrin. Results from these studies are summarized in Table 4-1.

In the aggregate, these studies show small but consistent excesses of lung cancer. Elevated relative risks appeared more frequently among applicators (greater than 1.0 in 5 of 5 studies ranging from 1.1 to 1.8) than among manufacturers (relative risks less than 1.0 in 2 of 4 studies). Information on smoking was lacking; but other tobacco-associated causes of death, such as emphysema and cancer of the mouth, pharynx, and esophagus, were not elevated. Relative risks for cancers of the lymphatic and hematopoietic systems, bladder, brain, and skin were reported less frequently, but, when available, tended to show slight excesses. Unfortunately, relative risks of less than 1.0 for some causes of death are often omitted from published reports. The risk of lung cancer increased with duration of employment as an applicator to about 3-fold in studies from Florida (Blair et al., 1983) and Germany (Barthel, 1981), but not in others (MacMahon et al., 1988; Wang and MacMahon, 1979). Blair et al. (1983) reported excesses in several application categories, including termite control (where chlordane was the chemical of choice), while MacMahon et al. (1988) did not see an excess among termite applicators. Time since first exposure appears to be a critical factor in these studies, since the lung cancer excess among applicators does not appear until 20 years after first exposure (Blair et al., 1983; MacMahon et al., 1988). Although the specific insecticides that may contribute to the lung cancer excess cannot be definitely identified, organochlorine insecticides seem likely candidates, because they were important chemicals in most of the time periods covered by these studies.

Studies of workers exposed to other classes of pesticides may also be profitably investigated, as indicated by the excess of cancer incidence found among arsenical manufacturers (Mabuchi et al., 1979) and grain handlers

TABLE 4-1. Relative Risks of Selected Cancers among Occupational Groups Exposed to Insecticides

					Relative Risk of Cancer and Number of Deaths[a]					
Reference	Occupation	Time Period	Major Insecticides	Number of Subjects Studied	Lung	Bladder	Brain	Skin	Lymphatic & Hema-topoietic	Leukemia
Mabuchi et al. (1979)	Arsenical manufacturers	1946-77	Arsenicals	1,050	1.7[b](23)	NA[c]	NA	NA	1.9(4)	NA
Wang and MacMahon (1979a)	Professional applicators	1967-76	Chlordane & others	16,126	1.2(21)	2.8(3)	NA	1.7(3)	0.7(5)	NA
Wang and MacMahon (1979b)	Chlordane manufacturers	1946-76	Chlordane/heptachlor	1,403	1.3(12)	NA	NA	NA	0.3(1)	NA
Morgan et al. (1980)	Pesticide workers	1971-77	Various	269	NA	NA	NA	5.7(4)	NA	NA
Barthel (1981)	Agricultural applicators	1948-72	Various	1,658	1.8[b](50)	NC[d](8)	NA	NC(19)	NC(12)	NC(3)
Ditraglia et al. (1981)	Manufacturers	1948-76	Organochlorines	2,141	0.8(20)[e]	NA	NA	NA	NA	NA
Blair et al. (1983)	Professional applicators	1965-77	Various	3,827	1.4(34)	1.6(3)	2.0(5)	1.3(2)	NA	1.3(4)

TABLE 4-1. Relative Risks of Selected Cancers among Occupational Groups Exposed to Insecticides (Continued)

Reference	Occupation	Time Period	Major Insecticides	Number of Subjects Studied	Relative Risk of Cancer and Number of Deaths[a]					
					Lung	Bladder	Brain	Skin	Lymphatic & Hematopoietic	Leukemia
Wong et al. (1984)	Manufacturers	1935-76	DDT, DBCP, TRIS, PBB	3,579	1.3(42)	0.9(3)	1.3(5)	NA	1.1(13)	1.9(9)
Ribbens (1985)	Manufacturers	1970-?	Aldrin, endrin dieldrin	233	NC(3)	NA	NC(1)	NA	NC(1)	NA
Schindell and Ulrich (1986)	Manufacturers[f]	1946-85	Chlordane/ heptachlor	800	0.9(13)	NA	NA	NA	NA	NA
Alavanja et al. (1987)	Grain workers	1968-83	Fumigants	1,114	1.1(90)	1.6(12)	0.9(5)	NA	1.5[b](32)	1.3(11)
McMahon (1988)	Termite applicators[g]	1967-84	Chlordane & others	16,124	1.4[b](99)	1.2(5)	NA	1.3(8)	1.0(23)	NA

[a] Deaths reported in parentheses.

[b] $p \leq 0.05$.

[c] Not available.

[d] Relative risk not calculated.

[e] Respiratory system cancer; 3 of 4 plants had excesses.

[f] One of the plants studied by Wang and MacMahon (1979b).

[g] An extended followup of cohort described by Wang and MacMahon (1979a).

(Alavanja et al., 1987). The significant excess of lung cancer among manufacturers of arsenicals (Mabuchi et al., 1979) is not surprising in view of the definitive epidemiologic evidence that arsenic is a lung carcinogen. Grain handlers have contact with various fumigants, including phostoxin, carbon tetrachloride, ethylene dibromide, ethylene dichloride, and malathion. No significant excess of lung cancer was seen in a proportionate mortality study of this group, but those employed in grain mills had a significant excess of lymphatic and hematopoietic cancer (Alavanja et al., 1987). In this study, however, it was not possible to evaluate specific pesticides, nor rule out the effect of other exposures such as grain dusts and mycotoxins.

Case-Control Studies with Information on Insecticide Exposure

A few case-control interview studies have reported cancer risks associated with exposure to specific insecticides (see Table 4-2). Investigations in which subjects were queried regarding contact with pesticides in general are not considered here. Soft-tissue sarcomas (STS) and cancers of the lymphatic and hematopoietic systems have been the major focus of these studies (Eriksson et al., 1981; Flodin et al., 1988; Hardell and Sandstrom, 1979; Hoar et al., 1986; Hardell et al., 1981; Woods et al., 1987; Zahm et al., 1987). Consistent excesses for STS, lymphoma and leukemia have been associated with reported contact with DDT (with odds ratios typically less than 2.0) and between STS and lymphoma with lindane (with odds ratios ranging from 1.9 to 6.0). Some relative risks for chronic lymphocytic leukemia (CLL) and non-Hodgkins lymphoma (NHL) from exposure to DDT were statistically significant. Although this may simply reflect the large number of cases in studies of these cancers, it is interesting that CLL and NHL are difficult to separate diagnostically and may, therefore, have similar etiologies. NHL was also associated with exposure to chlordane in two studies, but only one odds ratio was statistically significant. Most investigations either did not obtain information on other insecticides or did not report the findings, and many insecticides widely used today have not been evaluated in epidemiologic investigations.

These case-control studies suggest that the overall relative risk of cancer is small among farmers as a group. Greater risks, however, appear to occur among subgroups who have exposure to certain insecticides. More studies that focus on specific insecticide exposures and obtain information on duration, frequency, and level of exposure are needed to extend our knowledge in this area.

TABLE 4-2. Odds Ratios for of Selected Cancers from Potential Insecticide Exposure, Calculated in Case-Control Studies

					Odds Ratios and Number of Cases among Those Exposed^{a}									
							Specific Insecticides							
First Author (Date)	Cancer^{b}	Number of Cases/ Controls	Type of Interview	Years Covered	In Farmers	Insecticides	DDT	Chlordane	Lindane	Aldrin	Dichlorvos	Malathion	Nicotine
Hardell (1979)	STS	52/208	Mail/ phone	1970-77			1.2(4)						
Eriksson (1981)	STS	110/220	Mail/ phone	1974-78			1.3(7)		1.8^{c}(1)				5.4^{c}(3)
Woods (1987)	STS	128/694	In-person	1981-84	1.2(21)		1.1(4)	1.2^{d}					
Zahm (1988)	STS	133/948	Phone	1976-82	1.0(95)	1.3(50)	2.2(11)	—^{e}(0)	2.0(1)	—(0)	—(0)	1.2(2)	—(0)
Hardell (1981)	Lymphoma	169/338	Mail	1974-78			1.7^{c}(23)		—(0)				
Hoar (1986)	HD	121/948	Phone	1976-82	0.8(71)	0.8(38)	1.3(7)	—(0)	1.9(1)	1.9(2)	—(0)	0.8(2)	—(0)
Hoar (1986)	NHL	170/948	Phone	1976-82	1.4(133)	1.5(54)	1.3^{c}(6)	—(0)	6.0^{c}(3)	2.7^{c}(2)	6.7^{c}(1)	2.6^{c}(4)	—(1)

TABLE 4-2. Odds Ratios for of Selected Cancers from Potential Insecticide Exposure, Calculated in Case-Control Studies (Continued)

					Odds Ratios and Number of Cases among Those Exposed[a]								
							Specific Insecticides						
First Author (Date)	Cancer[b]	Number of Cases/ Controls	Type of Interview	Years Covered	In Farmers	Insecticides	DDT	Chlordane	Lindane	Aldrin	Dichlorvos	Malathion	Nicotine
Woods (1987)	NHL	576/694	In-person	1981-84	1.3[e](100)		1.8[f](31)	1.6(12)					
Flodin (1988)	CLL	111/431	Mail	1964-84	1.8[f](25)		6.1[f](6)						
Morris (1986)	Multiple myeloma						0.9(92)[g]						

[a] Numbers of cases among those exposed presented in parentheses.
[b] STS = Soft tissue sarcoma; HD = Hodgkin's disease; NHL = Non-Hodgkin's lymphoma; CLL = Chronic lymphocytic leukemia.
[c] Odds ratio not available in original paper; estimated here from percent exposed.
[d] Number of cases among those exposed not available.
[e] Estimate not possible from data provided.
[f] $p \leq 0.05$.
[g] Chlorinated hydrocarbons.

Cohort Studies of Herbicide-Exposed Populations

Cohort studies of herbicide-exposed populations have been conducted in Finland, Sweden, Denmark, West Germany, the United Kingdom, and the United States (see Table 4-3) (Axelson et al., 1980; Bond et al., 1988; Coggon et al., 1986; Kauppinen et al., 1986; Lynge, 1985; Ott et al., 1987; Riihimaki et al., 1982; Theiss et al., 1982; Wiklund et al., 1987 and 1988; Zack and Suskind, 1980). Occupations studied include wood workers, manufacturers of phenoxyacetic acids and the chemically related chlorophenols, applicators, railroad right-of-way sprayers, and manufacturing workers exposed to dioxins (contaminants of some herbicides) in industrial accidents. STS, NHL, multiple myeloma, and cancers of the stomach, prostate, and lung have been associated with herbicide exposure in more than one cohort study. Risks for cancer at rarer sites, however, have been based on small, statistically insignificant numbers (often only one death), making estimates unstable. The excess risk of STS appeared to increase with latency, duration, or level of exposure in three of the four investigations providing such information. Studies noting elevated risk of STS included manufacturers of chlorinated phenols in the United States (based on one case) (Ott et al., 1987), phenoxyacetic acids in Denmark (Lynge, 1985), and MCPA, also a phenoxyacetic acid, in the United Kingdom (also based on one case) (Coggon et al., 1986). The risk for lymphoma was elevated in three of five studies, but it did not appear to increase with latency or level of exposure, although numbers were small. The two cases of lymphosarcoma in the study by Bond (1988), however, occurred in the 2,4-D plant. Although these cohort studies of manufacturers involved populations with exposures to fewer pesticides than most studies of applicators or farmers, they were often limited by small numbers for the sites of greatest interest.

Several case reports of STS have been reported among workers exposed to dioxins in herbicide manufacturing plants. This association is suspect because in a review of the 7 "exposed cases," NIOSH concluded that only 2 had soft-tissue sarcomas linked with exposure to dioxin (Fingerhut et al., 1984). It is unclear whether this constitutes an excess, because the expected number was not presented.

Larger cohorts of manufacturers have been assembled by NIOSH in the United States, by IARC elsewhere, and the U.S. National Cancer Institute has initiated studies of herbicide applicators employed in lawn care and noxious weed control who come into contact with a limited number of chemicals. These cohort studies will provide valuable information in the near future.

TABLE 4-3. Relative Risks for Selected Cancers among Occupational Groups Exposed to Herbicides

First Author (Date)	Study Population	Years Covered	Specific Pesticides Used	Number of Subjects Studied	Basis for Exposure Estimate	Type of Cancer[b]	Relative Risk and Number of Deaths[a]			
							Overall Risk	By Duration	By Level	By Latency Period
Axelson (1980)	Railroad workers	1957-78	Phenoxy acids and amitrole	348	Job (phenoxy acids)	Stomach	2.2(3)			5.6(1)
						lung	1.4(3)			2.9(1)
Zack (1980)	Manufacturers	1949-78	TCP/Dioxin	121	All with chloracne from accident	Lung	1.8(5)			
						lymphatic & hematopoietic	3.4(3)			
Riihimaki (1982)	Herbicide applicators	1972-80	Chemical brush control (2,4-D & 2,4,5-T)	1,926	None	Stomach & esophagus				1.1(4)[d]
						prostate				1.8(2)[d]
						multiple myeloma				5.0(1)[d]
						lung				1.1(2)[d]
Thiess (1982)	Manufacturers	1953-76	TCP/Dioxin	74	Accident	Stomach	4.3(3)			
						lung	2.4(3)			
						STS	— (0)			
Lynge (1985)	Phenoxy manufacturers	1947-82	2,4-D, MCPA[e]	3,390[f]	Department	Stomach	1.3(12)			
						lymphatic	1.3(7)			1.3(4)[d]
						STS	2.7(5)			3.7*(4)[d]
						prostate	0.8(9)			

TABLE 4-3. Relative Risks for Selected Cancers among Occupational Groups Exposed to Herbicides (Continued)

First Author (Date)	Study Population	Years Covered	Specific Pesticides Used	Number of Subjects Studied	Basis for Exposure Estimate	Type of Cancer[b]	Relative Risk and Number of Deaths[a]			
							Overall Risk	By Duration	By Level	By Latency Period
Coggon (1986)	MCPA manufacturers	1947-83	MCPA, some organophosphates	5,784	Job title	Stomach	0.8(26)	0.9(10)[g]	0.7(7)[i]	0.7(12)[h]
						Lung	0.9(117)	1.0(46)[g]	1.0(43)[i]	0.9(63)[h]
						STS	1.1(1)	—(0)	2.8(1)[i]	2.0(1)
						Testis	2.4(4)	5.0(3)[g]	1.3(1)[i]	3.3(2)[h]
						NHL	0.4(2)	0.5(3)[g]	—(0)[i]	—(0)[h]
						MM	1.6(5)	2.7(3)[g]	1.9(2)[i]	2.2(4)[h]
						Leukemia	1.8(14)	1.4(4)[g]	2.1(6)[i]	1.5(6)[h]
						Nose	4.3(3)	—(0)[g]	12.5*(3)[i]	8.0(3)[h]
						Prostate	1.3(18)	1.4(6)[g]	1.5(5)[i]	1.4(10)[h]
Kauppinen (1986)	Woodworkers	1957-80	Arsenic, chromates, chlorophenols	3,805	Chlorophenol JEM by IH	Respiratory tract	0.6(3)		0.2(1)[i]	0.4(2)[d]
Ott[j] (1987)	Manufacturers	1940-82	Chlorinated dioxins	2,187	Job history and monitoring data	Resp. tract	0.8(23)		0.5(3)[k]	
						Stomach	1.6(6)		4.3(3)[k]	
						Prostate	1.9(8)		NA	
						NHL	1.9(5)		2.0(1)[k]	
						STS	2.5(1)		10.0(1)[k]	
						MM	2.0(2)		NA	
Wiklund (1987)	Licensed applicators	1965-82	72% phenoxy acids	20,245	Licensure	NHL	1.0(21)			1.2(16)[h]
						HD	1.2(11)			1.5(8)[h]

TABLE 4-3. Relative Risks for Selected Cancers among Occupational Groups Exposed to Herbicides (Continued)

							Relative Risk and Number of Deaths[a]			
First Author (Date)	Study Population	Years Covered	Specific Pesticides Used	Number of Subjects Studied	Basis for Exposure Estimate	Type of Cancer[b]	Overall Risk	By Duration	By Level	By Latency Period
Bond[j] (1988)	2,4-D manufacturers	1945-82	2,4-D	878	Quantitative, using job titles	Stomach	—(0)		NA	—(0)
						Colon	2.1(4)		NA	2.8(4)[h]
						Lymphatic & hemato poietic	2.0(5)		1.0(1)[i]	2.0(3)[h]
						Lympho-sarcoma	3.9(2)		NA	—(0)[h]
						Leukemia	2.2(2)		NA	3.6(2)[h]
						Other lymphoma	—(0)		NA	—(0)
						Lung	1.0(8)		1.4(5)[k]	1.1(7)[h]
Wiklund (1988)	Licensed applicators	1965-84	72% phenoxy acids	20,245	Licensure	STS	0.9(7)	1.2(6)[g]		1.0(4)[d]

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[a] Number of deaths presented in parentheses.

[b] STS = Soft tissue sarcoma; NHL = Non-Hodgkin's lymphoma; MM = Multiple myeloma; HD = Hodgkin's disease.

[c] $p \leq 0.05$

[d] 10-year latency.

[e] First licensed in 1965-66.

[f] Men only.

[g] ≥6 months duration.

[h] ≥15 years latency.

[i] Highest exposure level.

[j] These two studies partially overlap.

[k] Two highest exposure categories.

NA = Not available.

Case-Control Studies Focusing on Herbicide Exposure

There have been a number of investigations to assess the relationship between herbicides and cancer, particularly for STS and cancer of the lymphatic and hematopoietic systems. Publications have resulted from studies in Sweden, New Zealand, Italy, United Kingdom, and the United States (Table 4-4). This intense interest in herbicides was generated by a series of studies in Sweden that noted large (5-fold) risks for STS (Hardell and Sandstrom, 1979; Hardell et al., 1981) and Hodgkin's disease (HD) and NHL (Eriksson et al., 1981) among persons exposed to phenoxyacetic acids and chlorophenols. Risks for STS were elevated among those exposed to 2,4,5-T (OR = 17.0) and non-2,4,5-T phenoxyacetic acids (OR = 4.2) (Hardell et al., 1981). Although these studies have been severely criticized by some, they employed a sound study design. They were relatively small with limitations typical of case-control studies, but they were solid investigations overall and did not suffer from any clearly evident fatal flaw. Studies initiated following the Swedish reports have not been entirely consistent. Investigations in New Zealand found small, non-significant excesses of STS and multiple myeloma among persons exposed to herbicides (Pearce et al., 1986a; Pearce et al., 1986b; Pearce et al., 1987; Smith et al., 1984). However, multiple myeloma in farmers may be related to agents other than pesticides, if the results of a study in Sweden are relevant (Flodin et al., 1987). A study in Kansas found a slight overall excess of NHL among farmers using herbicides that rose to over 7-fold among those reporting use 20 or more days per year (Hoar et al., 1986). In this study, the risk of NHL was primarily associated with reported use of 2,4-D. No association was seen between the frequency of herbicide use and NHL in New Zealand (Pearce, 1989). Woods et al. (1987) found no overall association between NHL or STS and herbicide exposure in Washington, but did find more cases of NHL than expected among persons in several occupations where contact with phenoxyacetic acids was reported (farmers had a relative risk of 1.3, formulator-mixers had 1.5, and forest sprayers had 4.8). A recent study by Hardell and Erickson (1988) found STS to be associated with phenoxyacetic acids, but not chlorophenols. A 2-fold risk of STS occurred among women rice weeders in Italy who may have had contact with 2,4,5-T (Vineis et al., 1987). Single reports of elevated risk of ovarian mesothelioma (Donna et al., 1984) and cancers of the nasal passages and nasopharynx (Hardell et al., 1982) among herbicide-exposed populations have been published. Various herbicides have been implicated including 2,4-D, triazines, 2,4,5-T, and trifluralin, but most studies have focused on the broad categories of phenoxyacetic acids or chlorophenols.

TABLE 4-4. Odds Ratios for Selected Cancers from Case-Control Studies Focusing on Herbicides

					Odds Ratios and Number of Cases with Past Exposure^{b}						
					By Occupation		By Type of Exposure				
Reference	Cancer^{a}	Number of Cases/ Controls	Type Interview	Years Covered	Farmers	Foresters	Herbi-cides	Phenoxy Herbicides	Chloro-phenol	2,4-D	2,4,5-T
Hardell and Sandstrom, 1979	STS	52/208	Mail/ phone	1970-77				5.3^{c}(13)	6.6^{c}(7)		
Eriksson, et al. 1981	STS	110/220	Mail/ phone	1974-78				6.8^{c}(14)	3.3^{c}(11)	4.2^{c}(7)^{d}	17.0^{c}(7)
Smith et al. 1984	STS	82/92	Phone	1976-80	0.6(30)	0.9(7)	1.1(42)	1.3(21)	1.3(8)		1.0(12)^{e}
Hoar et al., 1986	STS	133/948	Phone	1976-82	1.0(95)		0.9(22)	1.4(15)		1.3(14)	1.4(4)
Smith and Pearce, 1986	STS	51/315^{f}	Phone	1976-80				0.7(6)			
Woods et al., 1987	STS	128/694	In-person	1983-85	1.2^{k}(21)			0.9(11)^{g}	0.9(13)^{g}		
Vineis et al., 1987	STS	68/158	In-person/ Mail	1981-83				2.7^{c}(8)^{h}			
Kang et al., 1987	STS	217/599	Phone	1975-80				0.8(45)^{i}			
Hardell and Eriksson, 1988	STS	54/490	Mail/ phone	1978-83				3.3^{c}(9)			
Hardell et al., 1982	NHL/ HD	169/338	Mail/ phone	1974-78				4.8^{c}(41)	4.3^{c}(50)		

TABLE 4-4. Odds Ratios for Selected Cancers from Case-Control Studies Focusing on Herbicides (Continued)

					Odds Ratios and Number of Cases with Past Exposure[b]						
					By Occupation		By Type of Exposure				
Reference	Cancer[a]	Number of Cases/ Controls	Type Interview	Years Covered	Farmers	Foresters	Herbicides	Phenoxy Herbicides	Chlorophenol	2,4-D	2,4,5-T
Olsson and Brandt, 1981	NHL of skin	5 cases	In-person	1978-81				4 cases			
Hoar et al., 1986	HD	121/948	Phone	1976-82	0.8(71)		0.9(28)	1.0(13)		1.0(13)	0.9(3)
Hoar et al., 1986	NHL	170/948	Phone	1976-82	1.4(133)		1.6(40)	2.2[c](24)		2.3[c](24)	1.2(3)
Pearce et al., 1986a	NHL	83/228	Phone	1977-81	1.3(40)	0.7(7)		1.1(51)	0.9(9)		1.4(14)[e]
Pearce et al., 1987	NHL	183/338	Phone	1977-81	1.0(81)	0.8(15)		1.0(44)	1.4(21)		0.9(25)[e]
Woods et al., 1987	NHL	576/694	In-person	1983-85	1.3[c](100)			1.2(48)[g]	0.9(55)[g]	0.7(19)	0.7(10)
Persson et al., 1989	NHL	106/275	Mail	1964-86	06(7)			2.7(6)			
Persson et al., 1989	HD	54/275	Mail	1964-86	1.0(6)			3.6(4)			
Hardell, 1981	Colon	157/541	Mail/ phone	1978-79				1.3(11)	1.8(6)		
Hardell, 1982	NPC	27/541	Mail/ phone	1970-79				2.0(3)[j]	9.9(5)[j]		

TABLE 4-4. Odds Ratios for Selected Cancers from Case-Control Studies Focusing on Herbicides (Continued)

					Odds Ratios and Number of Cases with Past Exposure[b]						
					By Occupation		By Type of Exposure				
Reference	Cancer[a]	Number of Cases/ Controls	Type Interview	Years Covered	Farmers	Foresters	Herbi- cides	Phenoxy Herbicides	Chloro- phenol	2,4-D	2,4,5-T
Hardell et al., 1982	Nasal	44/541	Mail/ phone	1970-79				1.8(5)[j]	4.3(4)[j]		
Donna et al., 1984	Ovarian mesothe- lioma	60/127	In-person	1974-80			4.4[c](18)				
Hoar et al., 1985	Colon	57/948	Phone	1976-82	1.6(47)		1.5(11)			2.0(6)	3.1(2)
Pearce et al., 1986b	Multiple myeloma	76/315	Phone	1977-81	1.7[c](43)	1.0(7)	1.3(45)	1.1(17)	1.1(6)		1.6(14)[k]

[a] STS = Soft tissue sarcoma; NHL = Non-Hodgkin's lymphoma; HD = Hodgkin's disease; NPC = Nasopharyngeal carcinoma.

[b] Number of cases with past exposure presented in parentheses.

[c] $p \leq 0.05$.

[d] Non-2,4,5-T.

[e] Sprayed gorse, blackberry, pasture, cereal peas.

[f] Additional cases and controls not included in Smith (1984).

[g] High exposure.

[h] For women only, no men with STS were exposed.

[i] Veterans who served in Vietnam.

[j] Crude rate ratio.

[k] Formulator/mixer.

The wide range for estimates of risk is unusual. It is not uncommon for epidemiologic studies to be inconsistent regarding small risks that can easily be accounted for by confounding, exposure misclassification, and biases. Factors that result in 5-fold differences between studies, however, are seldom seen. Even smoking would not typically be able to explain differences of this magnitude relating to lung cancer and occupational exposure (Axelson and Steenland, 1988).

The explanation for the differences in results from among studies is not clear, although there have been a variety of suggestions, including exposure misclassification, use of chemicals with different contaminants, different use practices, differences in susceptibility, design biases and limitations, and chance. Effect modification could also account for differences. Uncontrolled confounding seems an unlikely explanation, because no other common exposure factor is known with a comparable influence on risk, and several studies were able to adjust for known risk factors. Pesticides in different settings are used in a variety of situations and in association with differing factors. These other factors could modify the response observed. Exposure misclassification is a particularly difficult problem and undoubtedly occurs. The type of exposure misclassification is most likely, however, to be random and this would generally result in an underestimation of risk and depression of exposure-response gradients. Similarly, misclassification of cancer or inclusion of histologic types with different etiology would also bias risk estimates toward the null. This may be a particular problem for STSs, and to a lesser extent for NHLs, which are collections of tumors with different histologies. Thus, misclassification may be an explanation for the failure of some studies to show an association, but it is an unlikely explanation for the excesses. Case response bias is a form of misclassification that could lead to an overestimation of risk. Use of cancer controls in some studies indicate that this type of misclassification is an unlikely explanation.

The studies have used different methods to identify more heavily exposed subjects, which makes direct comparison difficult. Some have used years of exposure, some the usual number of days per year of use, and others, level of exposure. Improvement in exposure evaluation is needed, and biochemical measures should be included in future investigations to sharpen our efforts.

Cancer Risks from Non-Occupational Exposures to Pesticides

There has been a continuing concern over potential exposure to pesticides among members of the general population from residues in food and water (Durham, 1963; NRC, 1987), or from other non-occupational contacts. Few epidemiologic investigations, however, are available, exceptions being studies of acute leukemia (Lowengart et al., 1987; Shu et al., 1988) and brain cancer (Gold et al., 1979). The problems that must be faced to conduct epidemiologic studies of such exposures are immense and include accurate assessment of very small exposures and identification of unexposed persons for comparison.

Summary of Epidemiologic Results

Epidemiologic evidence suggests that, while there may be cancer risks associated with pesticide exposure, the risk varies by type of tumor and pesticide, with considerable inconsistency among studies. Excesses of lung cancer have been reported in most cohort studies of commercial pesticide applicators, where chlorinated hydrocarbon insecticides have been the chemicals of predominant use, but not among farmers. The apparently greater risk of lung cancer among structural applicators may be due to a greater potential for exposure because of application in enclosed spaces. The evidence for an association appears strongest for NHL where excesses are seen for insecticides and herbicides in both case-control and cohort studies. The probability of a causal association between herbicides and HD appears low since it has been reported in only one study. STSs pose the greatest difficulty in interpretation because of their inconsistent associations.

THE CARCINOGENIC POTENTIAL OF PESTICIDES AS BASED ON ANIMAL STUDIES

In the 1950s, animal studies came to be used increasingly for safety evaluation of chemicals although the recommended protocols were simple compared with those now used. A major focus was on the potential health effects of pesticide residues since residues of DDT in foods and in human fat had been detected as early as 1948 (Durham, 1963). Protocols for those substances tested in chronic studies followed FDA recommendations (1959) and used rats with controls and a minimum of three treated groups of 10 to 15 rats of each sex with a key objective being to establish a no-effect level as a basis of calculating an acceptable daily intake (ADI). Few, if any, pesticides were submitted for carcinogenicity testing on the scale implied by the 1959 FDA recommendations until about ten years later when the National Cancer Institute began its carcinogenicity screening program. Some of the data listed in Table 4-5 were obtained during this era.

TABLE 4-5. Results from Bioassays and Short-Term Tests (See Legend at End)

Active	Registrant Data					NTP Data				Other Data				IARC Classification			Ames	
Ingredient	MR	FR	MM	FM	NTP #	MR	FR	MM	FM	MR	FR	MM	FM	Overall	Human	Animal	Test	Comments
INSECTICIDES																		
ORGANOCHLORINES																		
Aldrin	-	-			021a	E	E	+	-					3	I	L	-	Mouse liver
Chlordane	-	-	+	+	008	-	-	+	+					3	I	L	-	Mouse liver
Chlordecone						+	+	+	+					2B	ND	S	-	Mouse and rat
Chlorobenzilate					075	E	E	+	+					3	ND	L	-	Mouse liver
DDT					131, ab&c	NT	NT	NT	NT	E	E	P	P	2B	I	S	-	Mouse liver, monkey negative
Dicofol					090	-	-	+	-					3	ND	L	-	Mouse liver
Dieldrin	-	-	+	+	021b,	-	-	+	+					3	I	L	-	Mouse liver
					022	-	-	NT	NT									
Endosulphan					062	I	-	I	-									
Endrin	-	-			012	-	-	-	-					3	ND	I	-	
Heptachlor					009	-	-	+	+					3	I	L	-	Mouse liver
Lindane					014	-	-	-	-	NT	NT	+	+	-	-	L	-	Mouse liver
Methoxychlor					035	-	-	-	-					3	ND	I	-	Mouse liver
Mirex	-	-								-	-			2B	ND	S		
Photodieldrin					017	-	-	-	-								+	
Toxaphene					037	E	E	+	+					2B	ND	S	+	Mouse liver

TABLE 4-5. Results from Bioassays and Short-Term Tests (Continued)

Active Ingredient	Registrant Data				NTP #	NTP Data				Other Data				IARC Classification			Ames Test	Comments
	MR	FR	MM	FM		MR	FR	MM	FM	MR	FR	MM	FM	Overall	Human	Animal		
ORGANOPHOSPHATES																		
Acephate	-	-	-	+														Mouse liver
Azinphos-methyl	-	-	-	-	069	E	-	-	-									
Coumaphos					096	-	-	-	-								-	
Diazinon					137	-	-	-	-								-	
Dichlorvos					010	-	-	-	-					3	ND	I	+	
Dichlorvos - 2nd study					342	+	+	+	+									Stomach, pancreas
Dimethoate					004	-	-	-	-								+	
Dioxathion					170,	-	-	-	-								+	
Fenthion					103	-	-	E	-								+	
Malathion					024, 192	-	-	-	-					3	ND	I	-	
Maloxon					135	-	-	-	-									
Methyl parathion					157	-	-	-	-					3	ND	L	+	
Parathion	E	-	NA	NA	070	E	E	-	-					3	ND	I	+	Spleen, thyroid
Phosphamidon					016	E	E	-	-								+	Spleen, thyroid
Tetrachlorvinphos	-	-	+	+	033	-	+	+	+					3	ND	L	-	Mouse liver, kidney, thyroid
Trichlorphon														3	ND	I		Rat thyroid

TABLE 4-5. Results from Bioassays and Short-Term Tests (Continued)

Active Ingredient	Registrant Data					NTP Data				Other Data				IARC Classification			Ames Test	Comments
	MR	FR	MM	FM	NTP #	MR	FR	MM	FM	MR	FR	MM	FM	Overall	Human	Animal		
OTHER INSECTICIDES																		
Aldicarb	NA	NA	-	-	136	-	-	-	-									
Calcium arsenate														1	S	L		
Carbaryl														3	ND	I		Mouse liver
Chlordimeform	E	E	+	+										3	ND	I		Hemangiomas
Copper arsenate														1	S	L		
Dichlorobenzene														3				
Lead arsenate														1	I	I		
Mexacarbate					147	-	-	-	-								NT	
Piperonyl butoxide					023	-	-	-	-					3	ND	I	-	
Piperonyl sulphoxide					124	-	-	+	-								-	Mouse liver
Sodium arsenate														1	S	L		
Sodium arsenite														1	S	L		

TABLE 4-5. Results from Bioassays and Short-Term Tests (Continued)

Active Ingredient	Registrant Data				NTP #	NTP Data				Other Data				IARC Classification			Ames Test	Comments
	MR	FR	MM	FM		MR	FR	MM	FM	MR	FR	MM	FM	Overall	Human	Animal		
HERBICIDES																		
PHENOXY																		
2,4,5-T	-	-						-	-					2B	L	I		
2,4-D	-	-						-	-					2B	L	I		
MCPA														3	-	ND		
OTHER																		
Acifluorfen	-	-	+	+														Mouse liver
Alachlor	+	+	-	+														Rat nasal, Rat stomach, Mouse lung
Amino-triazole						+	+	+	+									Rat thyroid, Mouse thyroid, Mouse liver
Amitraz	NA	NA	-	+														Mouse liver
Arsenic acid														1	S	L		
Asulam	+	-	-	-													+	Thyroid, adrenal
Chloramben					025	-	-	E	+								+	Mouse liver
Diallate														3	ND	L		
Fluometuron					195	-	-	E	-					3	ND	I	-	Integument
Glyphosate																		Testicular
Linuron	+	-	E	+														Rat adenomas, Mouse liver
Methanearsenic acid														1	S	L		
Metolachlor	-	+	-	-														Liver
Monuron					266	+	-	-	-					3	ND	L		Male rat Mouse kidney liver

TABLE 4-5. Results from Bioassays and Short-Term Tests (Continued)

Active Ingredient	Registrant Data					NTP Data				Other Data				IARC Classification			Ames Test	Comments
	MR	FR	MM	FM	NTP #	MR	FR	MM	FM	MR	FR	MM	FM	Overall	Human	Animal		
Nitrofen					026, 184	E -	+ -	+ +	+ +					2B	ND	S	+	Liver, circ. syst., pancreas
Ozadiazon	+	E	+	+														Liver
Sulfallate					115	+	+	+	+					2B	ND	S	+	Lung, mammary, stomach
Terbutryn	+	+	-	-													+	Mammary, thyroid
Trifluralin	+	+	-	-	034	-	-	-	+								+	NTP-Liver, lung, stomach kidney, bladder
FUNGICIDES																		
Anilazine					104	-	-	-	-								-	
Benomyl	-	-	+	+														Mouse liver
Captafol	+	+	+	+														Rat mammary, Rat kidney, Mouse liver
Captan	+	+	+	+	015	-	-	+	+					3	ND	L	+	Mouse intestine, Rat kidney
Chlorthalonil	+	+	+	+	041	+	+	-	-					3	ND	L	-	Rat kidney, Mouse stomach
Fenaminosulf					101	-	-	-	-								+	
Folpet	E	E	+	+														Duodenum
Fosetyl	+	-	-	-														Bladder, adrenal
Hexachloro-benzene														2B	I	S		
Maneb														3	ND	I		Inconsistent lung tumors in mice
o-Phenylphenol					301	-	-	-	-				3	ND	I			

TABLE 4-5. Results from Bioassays and Short-Term Tests (Continued)

Active Ingredient	Registrant Data				NTP #	NTP Data				Other Data				IARC Classification			Ames Test	Comments
	MR	FR	MM	FM		MR	FR	MM	FM	MR	FR	MM	FM	Overall	Human	Animal		
Pentachloro-nintrobenzene					061	-	-	-	-				3	ND	L	-		
Triphenyltin-oh					139	-	-	-	-				3	ND	I			
Zineb																		
OTHER																		
ANTU (rodenticide)															3			Mouse liver
DBCP*	+	+	+	+	028, 206	+	+	+	+					2B	I	S	+	Rat nose, liver, stomach, kidney
Dichloro-propene					269	+	+	I	+					2B	I	S		Forestomach, papilloma
EDB*					086, 210	+	+	+	+					2A	I	S	+	Multisite
Ethylene oxide*	+	+				NT	NT	+	+					2A	L	S		

MR = Male rat.
FR = Female rat.
MM = Male mice.
FM = Female mice.
ND = No data.
NT = Not tested.

L = Limited evidence.
S = Sufficient evidence.
I = Inadequate evidence.
E = Equivocal test.
- = Negative test.
+ = Positive test.
* = Fumigant.

Most of the carcinogenesis experiments conducted in the 1950s or earlier had small numbers of animals and in some cases were of limited duration. However, even with the relatively small number of two-year studies, certain problems with pesticides were evident at this early stage, such as possible liver hyperplasia/carcinogenesis with DDT and Aramite and thyroid tumors with aminotriazole (Durham, 1963). Bioassays in mice on a number of chemicals, including several pesticides, by the Bionetics Laboratory (Innes et al., 1969) under contract to NCI received considerable attention because of the large number of chemicals tested. They were limited, however, by the small number of animals per group and the short duration of exposure.

By the mid 1960s, the need was felt to increase the size of the two-year studies. Many studies were conducted by industry for product registration purposes and to establish an acceptable daily intake (ADI). It is not known precisely how many potential products have been eliminated because of animal toxicity or carcinogenicity, although this is known to have occurred. The studies now required for pesticide registration were outlined in 1978 (EPA) and expanded in 1979 (EPA, 1979). Combined protocols for chronic feeding and carcinogenicity assays are usually followed and currently call for the use of groups of at least 50 males and 50 females of two species exposed for two years at each dose level (with some caveats on survival). At least three dose levels and control groups are required. In practice, many studies have included more dose groups in order to add greater precision to no-observed-effect levels (NOELs) and ADIs for noncarcinogenic pesticides.

In addition to the studies carried out for registration purposes, many pesticides have also been studied under the NCI/NTP bioassay program. These studies are regarded as qualitative hazard identification studies and were not geared to end points such as determination of no-effect levels or dose-response relationships, although more recently more than two dose levels have been included. Other types of studies have also been conducted in academic laboratories, often using other kinds of protocols, e.g., skin-painting (Van Duuren et al., 1979). Thus, for many substances there are several carcinogenicity studies, but few of these are able to fulfill the current requirements of protocol and good laboratory practice. All positive studies may be used as evidence for carcinogenicity, but negative studies may simply be regarded as invalid and not utilizable for a weight-of-evidence analysis (see for example, the EPA Special Review on Dichlorvos, 1987).

In chronic animal studies, there are many potential end points which can be used for analysis. The current convention is to examine all organs and to tabulate any lesion which may be documented as hyperplasia, nodule, benign or malignant tumors. Thus, the tumor analysis is conducted organ by organ and by tumor type. Less emphasis is given to information such as time-to-tumor, tumor deficits, prognosis and patterns of effects.

Factors in experimental design and conduct that can affect tumor incidence have been documented on many occasions and include the feeding, caging, handling, number of animals per cage, effects on body weight, methods of feeding, and caging positions. Careful statistical design can reduce but not eliminate the importance of all these factors. All strains of laboratory rats and mice have characteristic patterns of tumors in control populations. Almost all animals may develop some types of tumor by the end of their lifetime. Thus, the interpretation of studies involves the consideration of many factors, including historical control data and other information.

Under the pesticide registration requirements that have been operative for the last twenty years, long-term studies in two species have been required if a food or crop tolerance was involved. Some of these studies may be regarded as invalid or ancillary data as judged by current standards of Good Laboratory Practice or Protocol requirements. In these cases a "data gap" would be signaled, but this reflects the need to repeat a study rather than the lack of any data *per se*. The repeating of studies to meet evolving regulatory requirements represents a major activity by the industry. It is emphasized that current EPA practices would not allow a pesticide with a positive tumor response to be registered without full consideration of the facts and additional review by the FIFRA Science Advisory Panel.

Analysis of Available Data

The information incorporated in Table 4-5 does not represent an exhaustive summary of all the information available, since this was beyond the scope of the present project. A starting point was a list of 53 pesticides identified by EPA as having carcinogenic potential from among the 289 pesticides with active food tolerances. To this list was added pesticides that have been tested by the National Toxicology Program (Ashby and Tennant, 1988; Haseman et al., 1987), and those that were listed by IARC (1986, 1987). Some limited data from studies provided to EPA by registrants are also included. It is emphasized that an absence of EPA data does not represent a lack of information, but rather that additional time and resources would be required by this Committee to review the material. Some compounds listed in Table 4-5 are no longer registered or used in the United States.

Table 4-5 does not include any consideration of potency or mechanism and was produced in order to evaluate trends. It may be desirable to expand the data base to allow a more detailed evaluation. A further issue is that there may be substantial differences in the conduct of studies, including exposure routes and animal strains used, although the later is standardized in the NTP database.

The pesticides tested in the NTP program were nominated because of some evidence of, or in consideration of, a possible positive response. Of the 51 pesticide compounds listed by NTP, 37% were negative in two species; whereas, 12% were positive in one species and 10% were positive in two species. On the other hand, of the pesticides listed in Table 4-5, IARC concluded that 43% were carcinogenic or probably carcinogenic in humans (overall ranking of 1 or 2). The remainder had equivocal results in one or more species or sexes.

Seven arsenicals are listed in Table 4-5 which are included in IARC Classification 1. Arsenic is a naturally occurring element that has been shown to be carcinogenic under conditions of heavy exposure.

The only three substances that produced multi-site tumors in two species are ethylene dibromide, dibromochloropropane, and ethylene oxide. Ethylene dibromide and dibromochloropropane were used as fumigants and are no longer registered for pesticide use. The materials were introduced into the market before carcinogenicity testing was a requirement and were later found to be carcinogenic to animals. IARC classifies ethylene oxide and EDB as 2A and DBCB as 2B. Several other pesticides included in Table 4-5 include tumor response that remain a matter of controversy. Among these are:

- **Thyroid Tumors:** Aminotriazoles and Ziram produce thyroid tumors which may result from a hormone effect. Hormones affect cancer in humans also. This emphasizes the importance of interspecies comparison of mechanisms to assist in determining whether a given response is likely to occur in humans.

- **Leukemias and Lymphatic Tumors:** Epidemiologic studies have suggested a relationship in humans between lymphoma and pesticides, particularly phenoxy herbicides, and between leukemia and ethylene oxide. In our brief review, with the exception of ethylene oxide, pesticides do not appear to cause this response in rats or mice.

- **Stomach Tumors:** Some pesticides, such as dichloropropene and dichlorvos, as well as several other substances tested by the NTP, have been associated with tumors of the rat forestomach when the test material was injected directly into the stomach by gavage, but not when the substances were incorporated into the feed or by inhalation exposure. There is disagreement as to whether these data provide a suitable diagnosis for human risk assessment.

- **Liver Tumors:** In the mouse, liver tumors are a common response to organochlorine insecticides such as DDT and to some other pesticides as well. No epidemiologic studies have demonstrated an equivalent response in exposed workers. In some cases, the response may be associated with rat thyroid tumors or a much reduced or equivocal result in the rat liver. The EPA Cancer Risk Assessment Guidelines (1986) discussed the significance of the mouse liver tumor response and defined some situations where the response might be considered a lower hazard. An important consideration is whether the substance possesses definite evidence of mutagenic activity, as do the nitrosamines, which also induce mouse liver tumors. In the absence of this positive information, substances such as DDT and phenobarbitone have been designated "non-genotoxic" or "epigenetic" carcinogens (Williams and Weisburger, 1986). Although the interpretation of mouse liver tumors has been at least a subject of considerable debate for years, little research has been focused on clarifying the issues of interpretation. Recent research on oncogenes that have been found in mouse liver tumors (Watanabe et al., 1987) and the unifying hypothesis of Trosko, which is discussed in the section on mechanisms, may clarify the situation.

- **Lung Tumors:** A few agents have increased the incidence of lung tumors in rodents, which may be important in light of the excesses of this cancer seen in some epidemiologic studies of pesticide applicators and manufacturers.

Dose-Related Issues

The purpose of long-term rodent studies is the detection of valid biologic evidence of the toxic or oncogenic potential of the specific substance being investigated. The goals of such studies, therefore, ought to be to maximize the sensitivity of the test without complicating the biologic interpretation of the data or encumbering the relevancy of the results for human risk assessments. The dose regimen of each study has an extremely important bearing on these goals, influences their interpretation, and can result in risk assessments of unproven value to society (Barr, 1987; Paynter, 1985). Two current dose concepts, the maximum tolerated dose and the putative "no oncogenic threshold," appear to conflict with the principle first articulated by

Paracelsus that is still fundamental to pharmacology, toxicology, and hazard assessment. The National Research Council has restated this axiom as: a chemical--any chemical--is a poison only as a consequence of the quantity with which the host must deal (NRC, 1975). The controversy engendered by this apparent conflict and the debate regarding the appropriateness of both concepts in the hazard identification and the risk assessment processes may never be resolved to everyone's satisfaction, yet the issues must be confronted anew each time a long-term study is interpreted (Maronpot, 1985; Weisburger and Williams, 1984).

Historically, the maximum tolerated dose (MTD) concept arose in long-term oncogenicity screening studies employing limited dose regimens and relatively small numbers of animals. The intent in using the MTD was to maximize the likelihood of observing an oncogenic response by administering as high a dose of the chemical as feasible in order to determine, in a qualitative sense, whether or not the administered substance had oncogenic potential.

Proponents justify the need for an MTD by implying that maximum sensitivity is to be accomplished by using as large a dose as possible that will not kill so many animals as to invalidate the test (NTP, 1984; Office of Science and Technology, 1985). Opponents and critics of the MTD do not dismiss the notion that present long-term studies may be insensitive: the lowest incidence of tumors detectable with statistical reliability ranges from 7 to 10% (Rodricks and Taylor, 1983). Nevertheless, they are concerned about the biologic implication of very high doses administered over prolonged periods (Clayson, 1987). These concerns have been summarized as follows: a) the underlying biologic mechanisms that lead to the production of cancer may change as the dose of the carcinogen changes; b) current methods of estimating an MTD for use in an experiment do not usually take these mechanisms into account; c) the biologic mechanisms at work under conditions of actual human exposure may be quite different from those at work at or near an MTD; and, therefore, d) observations at or near an MTD may not be qualitatively relevant to conditions of actual human exposure (Rodricks and Taylor, 1983).

Various definitions of the MTD have been proposed (Friedman, 1973; Gori, 1980; Roe, 1983). It has been described as the highest dose that can be given that does not cause a significant decrease in survival from effects other than carcinogenicity (Hamm, 1985), or the maximum dose that an animal species can tolerate for a major portion of its lifetime without significant impairment of growth (Rodricks and Taylor, 1983). The EPA characterizes the MTD as a dose which produces signs of minimal toxicity that do not compromise the biologic interpretability of the observed responses and, in a negative sense, by producing many effects it should not produce. For example, the highest dose should not: a) alter survival in a significant manner due to effects other than tumor production; b) cause a body weight decrement from concurrent control values of greater than 10-12%; c) exceed 5% of the total diet because of potential nutritional imbalances caused at higher levels; or d) produce severe toxic, pharmacologic, or physiologic effects that might shorten duration of the study or otherwise compromise the use of study results in risk assessment (Paynter et al., 1985). The inability to state the precise meaning or describe the nature or basic qualities of the MTD, except by negation, contributes to the controversy.

Proponents of the no-oncogenic-threshold concept argue that cancer results from a permanent, replicable defect initiated in a single cell by reaction of a chemical with one or more critical receptors. Once this has occurred, the cell may remain dormant for many years before expressing a discernible effect. Proponents of this concept also refer to evidence that exhaustive experiments on radiation-induced neoplasia have not revealed any thresholds within the realm of statistical reliability (Gehring et al., 1976). A further concern is that even if oncogenicity thresholds do exist, it is impossible at present to discern them with any degree of reliability.

The evidence supporting a threshold concept (for toxic effects or carcinogenicity) has been discussed by Gehring and Blau (1977). Essentially, the argument assumes that chemical carcinogenesis is a multistage process involving: a) exposure, absorption, distribution, activation, deactivation, and elimination of the chemical *per se*, or products formed from it; b) interaction with critical receptor sites leading to molecularly transmittable products; and c) survival and proliferation of transformed cells to clinical cancer. Interference with any of these processes may constitute a threshold.

An example of a threshold effect in an experimental system is that concerning thyroid follicular cell neoplasia (Hill et al., 1989; Paynter et al., 1988) In this system, if the pharmacologic effects, thyroid hypertrophy, and hyperplasia are prevented or controlled with the feedback system, follicular cell neoplasia does not develop. These data also suggest that some degree of hypertrophy or hyperplasia of the thyroid gland is tolerated within the oscillation of the feedback system without induction of neoplasia. Finally, evidence that treatment of human hyperthyroidism with goitrogens is without appreciable risk of neoplasia (Hayes, 1982) is also indicative of the operation of a threshold for the induction of thyroid/pituitary neoplasia. When data demonstrate an ordered linkage of the steps (thyroid blockade, continuous thyroid stimulating hormone release, thyroid hypertrophy/hyperplasia, nodularity, adenoma/carcinoma), then the threshold for an earlier step automatically becomes a threshold for the entire chain of steps. New models are being developed (Sielkin, 1987) in attempts to clarify the difficult problem of establishing the probable existence of thresholds.

Summary and Conclusions

Animal studies have provided a very valuable mechanism for screening pesticides for carcinogenic activity. The state of the art for both the development of new pesticides and carcinogenicity testing has moved in parallel. The regulatory process has removed several probable or possible human carcinogenic pesticides from the market place, and in the case of the remaining products it is considered very important that further evidence or possible mechanisms be obtained in order to assist in the risk assessment process. Such information on mechanisms may help explain epidemiologic results that are not seen in the animal models, as is the case for the phenoxy acids.

MECHANISMS OF ACTION OF CARCINOGENESIS

The Nature of the Carcinogenic Process

Carcinogenesis is a multi-event process in both experimental animals and humans. Operationally, two principal steps have been identified, which are referred to as initiation and promotion (Berenblum, 1974; Boutwell, 1974) or neoplastic conversion and neoplastic development (Williams and Weisburger, 1986). Initiation or neoplastic conversion appears to be an irreversible step in which the normal cell is altered or transformed into a neoplastic cell by the carcinogen. The molecular mechanism underlying this event is not yet fully understood, but for many years has been considered to involve somatic mutation. Most of the carcinogens that act as initiating agents form electrophilic reactants and hence are mutagenic or otherwise genotoxic in short-term tests. Such carcinogens have been referred to as primary, genotoxic, or DNA-reactive carcinogens (Williams and Weisburger, 1986).

Cells normally contain a certain class of genes, called oncogenes, which appear to control cellular growth. Activation of an oncogene may represent one event in carcinogenesis. Mutation or gross DNA rearrangement such as chromosome translocation or gene amplification may occur in these genes and thereby result in the loss of their capacity to regularize cell replication. The number of oncogenes that must be activated in the multistep process of carcinogenesis is unclear at present, although concerted expression of at least two oncogenes, *ras* and *myc*, are needed for the transformation of cells in culture (Land et al, 1983).

Ras-oncogenes acquire transforming properties by qualitative and quantitative mechanisms. Most *ras*-oncogenes owe their transforming properties to single point mutations within their coding sequences (Bos, 1988). However, increased expression (10- to 100-fold) of non-mutated *ras*-genes can also induce certain characteristics of the malignant phenotype. Mutations in *ras*-oncogenes have been localized in codons 12, 13, 59 and 61. In human and in carcinogen-induced animal tumors, mutations occur most often at codons 12 and 61. In chemically-induced rodent tumors, the mutations have been consistent with the known alkylating patterns of DNA-reactive carcinogenesis.

Many approaches have been developed to assess the genetic effects of suspected carcinogens. Findings of mutagenicity of many carcinogens gave rise to the widely held paradigm that carcinogens are mutagens. This paradigm has strongly influenced the strategies for carcinogen detection and interpretation of carcinogenesis bioassays. While direct measurement of changes in the genome would seem to provide evidence of chemical mutagenicity, even these techniques are not infallible. For example, the mitogenic activity stimulated by the chemicals could have led to the production of spontaneous mutations arising during replication. Nevertheless, this type of analysis might be informative for compounds such as pesticides, many of which are largely negative (total weight of evidence) in genotoxicity tests, but which are carcinogenic in long-term animal bioassays (see Table 4-6).

A variety of short term tests have been studied for their ability to detect carcinogens (Tennant et al., 1987). Many of these assays (Ames Salmonella test, sister-chromatic exchange, unscheduled DNA synthesis, DNA strand breaks, dominant lethal tests, etc.) are, in one way or another, indirect measures of mutagenesis. Others, such as the mammaliam gene mutation assay and the chromosomal aberration assay measure mutation directly. Many of these short-term assays have one or more limitations and potential artifacts that can make interpretation of the results difficult (Trosko, 1984; Trosko and Chang, 1988).

The qualitative and quantitative changes observed in the oncogenes of tumors produced by the studied chemical also may be used to argue that the carcinogen acts via a genotoxic mechanism. Comparison of patterns of oncogene activation in "spontaneous" versus chemically-induced mouse liver tumors has shown, for instance, that chemicals (furan, furfural) that are non-mutagenic in the Ames Salmonella assay are able to induce activating mutation in different codons of the *ras*-gene (Reynolds et al., 1987).

Table 4-6. Promoting and/or Inhibiting Studies with Pesticides

Agent	Organ	Species	Reference
DDT	Liver	Rat	Peraino et al., 1975
	Liver	Rat	Nishizumi, 1979
	Liver	Mouse	Williams & Numoto, 1984
Chlordane	Liver	Mouse	Williams & Numoto, 1984
Hexachlorobenzene	Liver	Rat	Pereira et al., 1982
Heptachlor	Liver	Mouse	Williams & Numoto, 1984
Lindane	Liver	Rat	Pereira et al., 1982

Following the generation of a neoplastic cell, this cell must achieve progressive growth in order to form a tumor. It is apparent that initiated neoplastic cells can survive surrounded by normal cells until liberated for progressive growth. This step has been referred to as promotion or neoplastic development. Promotion appears to be an interruptible, if not reversible process (Pitot et al., 1978; Trosko and Chang, 1985). Promotion can be achieved through a variety of mechanisms, including cytotoxicity, inhibition of cellular differentiation, immunosuppression, mitogenesis and inhibition of cell-to-cell communication (Williams, 1984). These effects, unlike those involved in initiation, do not appear to depend on genetic change.

It is now apparent that chemicals acting at this step can lead to an increase in cancer. One possible explanation is that they facilitate development of cryptogenic neoplastic cells. To distinguish such chemicals from the genotoxic type that produces genetic change, they have been referred to as secondary or epigenetic carcinogens (Williams and Weisburger, 1986).

In the evolution of a tumor, qualitative changes in the properties of the tumor or cells have been referred to as progression. This stage may involve additional genetic changes.

The following sections describe findings from studies designed to elucidate the mechanisms by which pesticides might exert their carcinogenic effects.

In Vitro Studies and Genetic Toxicity

The literature is replete with studies which have been designed to determine if specific pesticides are mutagenic (Ashwood-Smith, 1981; Garrett et al., 1986; Glatt et al., 1983; Klopman et al., 1985; Moriya et al., 1983; Sandhu and Waters, 1980; Sauers et al., 1982; Shirasu et al., 1976; Sram et al., 1981; Whorton et al., 1983; Woo, 1983). No doubt some pesticides are mutagenic; for example, the potent genotoxic carcinogen 2-acetylaminofluorene was originally developed as a pesticide. However, in light of the aforementioned issues and recent research, the weight of evidence seems to indicate most of the thoroughly studied pesticides are not mutagenic (even given the limitations of the genotoxicity assays stated above), and hence, it might be more helpful to examine non-mutagenic mechanisms by which these chemicals could act.

Although some pesticides have been shown to be positive in one or more *in vitro* short-term tests for genotoxicity, in light of the potential artifacts associated with these assays, mutagenic activity must be confirmed, preferably using *in vivo* assays. Only then can the mutagenicity of a chemical be assessed adequately. Despite the carcinogenicity of many pesticides, some will ultimately be found to be nonmutagenic. It is important to consider nongenotoxic mechanisms by which agents may be carcinogenic. Two possible nongenotoxic mechanisms of carcinogenesis are the inhibition of gap-junctional intercellular communication, and the induction of peroxisome proliferation.

Pesticides as Inhibitors to Gap-Junctional Intercellular Communication

Many pesticides inhibit gap-junctional communication in a dose-dependent manner showing a no-effect level. This cellular end point is a membrane-dependent phenomenon and a reversible process. Since intercellular communication has been associated with the regulation of cell proliferation, differentiation, or the function of the differentiated cell (Loewenstein, 1979, 1987; MacDonald, 1985; Pitts et al., 1986), inhibition of this basic process needed for tissue homeostasis could lead to potential toxic end points, depending on important circumstances (Trosko et al., 1984).

If a pesticide inhibits intercellular communication during critical periods of organogenesis, embryotoxicity or teratogenesis could result (Trosko et al., 1982). If chronic inhibition of intercellular communication occurs in a tissue which has been initiated by a mutagen or carcinogen, then tumor promotion could occur (Trosko et al., 1983). If inhibition of intercellular communication in the central nervous system occurs, neurotoxic effects could result (Trosko et al., 1987, 1988). If pesticides inhibit maturation and differentiation of germ cells in the ovum or testes by inhibiting gap junctions known to exist in these tissues (Gilula et al., 1976; Schultz, 1985), then reproductive toxicities would occur.

DDT, aldrin, dieldrin, toxaphene, chlordane, heptachlor, heptachlor epoxide, lindane, kepone, mirex, dicofol, endrin, have all been shown to have some toxic effects (carcinogenicity, neurotoxicity, etc.). All these chemicals have now been shown to inhibit gap junctional intercellular communication (Trosko and Chang, 1988; Williams, 1983b). Several of these same chemicals were simultaneously tested for their ability to induce DNA damage or point mutations in the same systems in which they inhibited intercellular communication (Maslansky et al., 1981; Tsushimoto et al., 1983; Williams, 1981) in those cases where the pesticides have been tested in an initiation/promotion assay. Clearly, chemicals, such as DDT, have been shown to be promoters of carcinogenesis (Table 4-6) (see **Tumor Promotion Studies** below).

Some of the major implications of these findings would indicate that the "threshold concept" would be appropriate to consider for these pesticides. Mitigating factors, such as persistence of the chemicals in the environment, bioaccumulation, exposure of pregnant women, and possible synergisms or antagonisms with other endogenous or exogenous chemicals (Aylsworth et al., 1989; Warngard et al., 1987) have to be considered.

In conclusion, it is possible that some pesticides might produce toxic effects in animals as a consequence of their ability to inhibit intercellular communication. Moreover, because of numerous factors (concentration dependence, species, cell type, developmental stage, etc.) it may be that a no-threshold-level model is not appropriate to assess the risk from all pesticides (see **Dose-Related Issues**). In future studies, the biologic aspect of the multistage nature of carcinogenesis should be considered and this approach more rigorously applied to investigations in humans.

Tumor Promotion Studies

Tumor promoters are agents that were originally defined from studies on skin carcinogenesis (Berenblum et al., 1974) as agents acting to facilitate the development of latent tumor cells into actual tumors. Mechanistically, promoters operate after the initiating process in which normal cells are converted to tumor cells. On this basis, it was assumed that promoters would not be carcinogens and in most early studies on skin tumor promotion in which the promoter by itself was given in limited amounts or for limited duration, no tumors occurred. However, it gradually became evident that, in experimental situations, exposure to promoters alone could produce an increase in tumor yield. The underlying mechanisms are not known, but several possibilities exist.

In increasing tumors, promoters could simply be enhancing the development of cryptogenic neoplastic cells into tumors. This possibility is supported by observations that promoters have epigenetic membrane effects which impede cell-to-cell communication (see ***In Vitro* Studies and Genetic Toxicity**) and release cryptogenic neoplastic cells from regulatory control by surrounding normal cells. Another possibility is that promoters have some weak initiating activity. Support for this latter hypothesis has been sought in studies of genetic effects of promoters, but the evidence to date is not very supportive.

In studies of promoting activity, the test agent is administered after administration of a genotoxic carcinogen that affects the organ where promotion is being studied. The incidence of tumors is compared to that produced by the carcinogen alone. In the early work on skin tumor promotion, the promoter was also applied in one group before the carcinogen to demonstrate that no enhancement occurred under these conditions. In subsequent studies of promotion in other organs, reverse administration has rarely been done (Williams and Furuya, 1984). Without such information, it is not possible to determine whether enhancement represents promotion or a syncarcinogenic action (Williams, 1984).

In 1975, Peraino and coworkers (1975) reported an enhancing effect of DDT on liver carcinogenesis in the rat. Subsequently, several other pesticides have been shown to promote liver carcinogenesis in rats or mice (Table 4-6). Most of these studies have measured tumor incidence as the end point, but with the demonstration that liver tumor promoters enhance the development of preneoplastic lesions in the liver (Pitot et al., 1978; Watanabe et al., 1978), enhancement of altered foci in the liver has been used in some studies (Pereira et al., 1982). Interestingly, DDT is reported to be inactive as a liver tumor promoter in hamsters (Tanaka et al., 1987). The liver may be especially susceptible to promotion, since reports of promoting effects of pesticides in organs other than the liver have not been made. DDT, chlordane, or heptachlor were ineffective in promoting cancer in mouse forestomach or lung (Williams and Numoto, 1984).

Peroxisome Proliferation Studies

Peroxisome proliferation may represent another non-genotoxic carcinogenic mechanism. The term "peroxisome proliferator" was introduced by Reddy et al. (1975) to designate a xenobiotic that induces proliferation of peroxisomes in liver cells. The finding that compounds capable of inducing peroxisome proliferation in liver also induce hepatocellular carcinomas in rodents has led to the suggestion that peroxisome proliferators act as a novel class of non-mutagenic carcinogens (Reddy et al., 1980). They induce a similar type of response consisting of hepatomegaly, proliferation of peroxisomes, and induction of several hepatic enzymes, particularly those of the peroxisomal fatty acid beta-oxidation system. Proliferation is also observed in the kidneys of the rodents. Primates, however, may be less sensitive to chemically-induced peroxisome proliferation than rodents (Reddy et al., 1983, 1984).

The lack of mutagenicity of peroxisome proliferators in prokaryotic and eukaryotic test systems (Rao et al., 1987) led to the hypothesis that their carcinogenicity was related to biologically active products of the proliferated peroxisomes rather than to the direct DNA-damaging effect of the chemical (Reddy and Lalwani, 1983; Reddy et al., 1982). This hypothesis suggests that induction of peroxisomal beta-oxidation increases the hydrogen peroxide levels in the liver, causing oxidative stress in the cells. However, evidence that peroxisome proliferation and the resultant oxidative stress leads to DNA damage *in vivo* is lacking (Elliot et al., 1987).

In the initial step of the peroxisomal fatty acid *beta*-oxidation sequence system, fatty acyl-CoA oxidase donates electrons to molecular oxygen, producing hydrogen peroxide. The activity of fatty acyl-CoA oxidase in liver is increased 5- to 20-fold by treatment with peroxisome proliferators, whereas the activity of catalase, which degrades hydrogen peroxide in the peroxisome, is enhanced only 1- to 3-fold (Reddy and Lalwani, 1983). Some peroxisome proliferators have also been reported to inhibit the activity of glutathione peroxidase (Furukawa et al., 1985) which degrades hydrogen peroxide in the liver cytoplasm. There is a recent report that the inducibility of peroxisomal enzymes is under genetic control (Butler et al., 1988a).

Several researchers have reported damage, including increases in lipofuscin and conjugated dienes, in livers of rats treated for more than six months with peroxisome proliferators (Goel et al, 1986). These increases are presumably due to increased lipid peroxidation in livers of treated rats and are consistent with the role of reactive oxygen species in peroxisome proliferator-induced cancer. However, it has not been established whether the observed oxidative damage and carcinogenesis is due to excess hydrogen peroxide production by the peroxisomes.

Several reports indicate that chlorophenoxy herbicides (2,4,5-T, 2,4-D and MCPA) act as peroxisome proliferators in rats, mice, and Chinese hamsters (see Table 4-7) and there is preliminary evidence that a mixture of chlorophenols used in wood preservation increases the number of peroxisomes in the liver. Further, 2,4,5-T and 2,4-D have been shown to inhibit intercellular communication in Chinese hamster cell *in vitro* at non-cytotoxic levels (Rubenstein et al., 1984). Other pesticides, such as tridiphane and lactofen (Butler et al., 1988b), clearly induce proliferation of peroxisomes.

Phenoxyacetic acid herbicides 2,4-D, 2,4,5-T and MCPA are structurally closely related to the hypolipidemic drug clofibrate (ethyl-2-(4-chlorophenoxy)-2-methylpropionate, a well-established peroxisome proliferator.

TABLE 4-7. Summary of the Results in the Literature of Pesticides Causing Peroxisome Proliferation

Pesticide	Peroxisome Proliferation		HL[a]	HM[b]	Cat[c]	Beta Oxide[d]	CATr[e]	Animal	Reference
2,4-D	s[f]+	n[g]+						CH	Vainio & Linnainmaa, 1982
						+ +[j]	+ +	Rats	Vainio et al., 1983a
	s+	n+	-[h]		+ +		+ +	Rats	Vainio et al., 1983b
			+[i]	-	-	+	+	Rats	Kawashima et al., 1984
					+	+ +		Rats	Katoh et al., 1984
						+ +		Rats	Hietanen et al., 1985
				+	-	+ +	+ + +[k]	Mice	Lundgren et al., 1987a
					-	+ +	+ + +	Mice	Lundgren et al., 1987b
2,4,5-T			+	+	+	+ + +	+ + +	Rats	Kawashima et al., 1984
				+	+	+ + +		Rats	Katoh et al., 1984
				+	-	+ +	+ + +	Mice	Lundgren et al., 1987a
					-	+ +	+ + +	Mice	Lundgren et al., 1987b
MCPA	s+	n+						CH	Vainio & Linnainmaa, 1982
			+	+	+ +	+ +	+ +	Rats	Vainio et al., 1983a
	s+ + +	n+ + +	+ +		+ +		+ +	Rats	Vainio et al., 1983b
						+ +		Rats	Hietanen et al., 1985
Tridiphane	n+ +		+ +	+ +	-	+ +	+ +	Mice	Moody & Hammock, 1987

TABLE 4-7. Summary of the Results in the Literature of Pesticides Causing Peroxisome Proliferation (Continued)

Pesticide	Peroxisome Proliferation	HL[a]	HM[b]	Cat[c]	Beta Oxide[d]	CATr[e]	Animal	Reference
Lactofen	n++		++	++	+++		Mice	Butler et al., 1988b
	n++					++[m]	Rat hepatocytes[l]	Allen et al., 1988
Lactofen metabolites	Less proliferation				++		Rat hepatocytes[m]	Allen et al., 1988
KY-5[n]	s- n++		++				Rats	Mustonen et al., 1989

[a] Hypolipidemia.
[b] Hepatomegaly.
[c] Catalase.
[d] CN-insensitive palmitoyl-CoA oxidation.
[e] Carnitine acetyltransferase.
[f] Mean size of peroxisome.
[g] Mean number of peroxisomes.
[h] No effect.
[i] Slight effect.
[j] Clear effect.
[k] Overwhelming effect.
[l] Personal communication.
[m] *In vitro*.
[n] Commercial mixture of chlorophenols.

Despite the concerted research effort directed toward assessing the relevance of peroxisome proliferation in various species, there is no consensus as to its role in the etiology of human carcinogenesis. It can be postulated that rodent liver cells are more readily inducible by specific chemicals or that they are more sensitive to the hydrogen peroxide produced by the peroxisomes than human liver cells. Unfortunately, it is not possible to resolve this question at this time.

EXPOSURE

Exposure Assessment for Toxicology Studies

In most countries, pesticides are subject to premarket regulation, which means that they have been tested in several species of animals for varying periods of time to assess their potential to cause acute and chronic effects. In these controlled animal studies, the oral (gastrointestinal) route of exposure is the one used predominantly in the chronic toxicity/oncogenicity study, andthe animals are dosed daily for 18 months (mice) and two years (rats) (OECD, 1981). The incidence of tumors is then related to the daily lifetime dose.

When extrapolating the animal data to humans, it is presumed that humans are at least as sensitive to the chemical as the test animals and the route and duration (lifetime) of exposure are comparable for humans and test animals. These assumptions seem reasonable when evaluating cancer risks from pesticide exposure via residues in food or drinking water, where accurate measurement of the pesticide residue is available and where consumption patterns are known. These assumptions may be less valid where the exposure occurs from direct contact during pesticide application (mixers, loaders, applicators), in handling material with residues (harvesters, pruners, field workers), or through inadvertent exposure after someone else has used the product (bystander exposure). Under these circumstances, the route of exposure is predominantly dermal, although exposure also occurs through inhalation. It cannot be assumed that exposure and dose are equivalent. Many pesticides are poorly absorbed percutaneously and an estimate of contact exposure could be much higher than the actual absorbed dose. In addition, metabolism, delivered dose to the target site, and excretion may all be affected by the route of exposure (Franklin, 1989).

Another difficulty that arises when using animal carcinogenicity data for estimating human risk is the disparity in frequency and duration of exposure. Animals are exposed daily throughout their lifetime. This is rarely the case in humans who are more likely to be exposed intermittently for several days at a time with varying periods of time between exposures (Franklin, 1985). Problems arise when this short-term human exposure is then amortized to provide an estimate of lifetime exposure. Current quantitative risk estimation methods are unable to handle this problem.

Exposure Assessment for Epidemiology Studies

The distribution of exposure is a major factor affecting the distribution of a disease or the health effect in a human population. Quantitative exposure assessment in epidemiologic studies, however, is difficult, since pesticide workers generally have been exposed to a wide variety of chemicals, and the most relevant exposures may have occurred years in the past. Often qualitative or semi-qualitative estimates of exposure are the best that can be obtained.

There are several ways to estimate exposure, starting from the most general and qualitative ways to the more specific and quantitative:

1) Population groups potentially at risk can be identified through qualitative estimates made from:

   (a) inventories of quantities used from private, federal, state/provincial sources;

   (b) application rates allowed by regulatory agencies for registered uses;

   (c) recommendations on frequency and use of products in combination, as outlined in state/provincial spray calendars; and

   (d) information from agricultural chemical associations in the U.S. and Canada on retail sales.

2) Semiquantitative estimates (high, mid, low exposure) for specific groups may be based on:

   (a) surveys that ask specific questions on target crops, chemicals used, quantities used and application techniques (i.e., type of equipment, tank size, frequency of application, duration of application, protective equipment/clothing worn, personal hygiene); and

   (b) exposure models.

3) Quantitative estimates of exposure may be based on actual measurements, including:

   (a) data from specific studies of individual products (there are usually limitations due to small number of replicates and inherent difficulties in conducting such studies; however, these studies can provide quantitative estimates for exposure categories; additional information should be obtained from industrial hygiene monitoring, biochemical monitoring, and pharmacokinetic data on absorption, excretion, and metabolism to estimate delivered dose to the target site, rather than simply estimating dermal contact exposure and correcting for absorption); and

   (b) development and use of a generic database.

The latter approach assumes that the various types of application can be grouped into exposure scenarios. It is likely that exposure is similar for workers using similar types of equipment, similar use rates and similar formulation types--that is, workers using orchard air-blast equipment would likely be exposed to similar amounts of pesticide per kg of pesticide used, even though the specific chemical could be different (i.e., parathion, guthion, carbaryl). Backpack sprayers would receive a different amount due to the vastly different mode of application. This approach may be the most useful for epidemiology studies, since it would allow estimates of relative levels of exposure for situations where actual measurement might be impossible.

Summary

Epidemiologic studies of occupational groups exposed mainly to insecticides have noted small, but consistent, excesses of lung cancer (less than 2-fold among workers with likely exposure to organochlorine insecticides). Less common cancers, such as those of the bladder, brain, and lymphatic and hematopoietic systems, are less consistently elevated in these cohorts. Case-control studies of soft-tissue sarcoma, leukemia, and lymphoma have noted excesses among farmers and foresters exposed to organochlorine insecticides, particularly DDT, chlordane, and lindane. Positive findings from DDT and chlordane are available from animal bioassays, but both are negative in the Ames salmonella test. Several organochlorine insecticides have been shown to inhibit intercellular communication and to act as tumor promotors.

In several case-control studies and cohort studies of herbicide manufacturers and applicators, associations occurred between phenoxyacetic acid herbicides and soft-tissue sarcomas and non-Hodgkins lymphoma. According to currently available data, phenoxyacetic acids have not been reported to be animal carcinogens, but they can act as peroxisome proliferators in animals and, as such, may play some role in carcinogenesis.

In animals, most carcinogenic pesticides affect a limited spectrum of sites. A few such as captafol, DBCP, tetrachlorvinphos, trifluralin, and ethylene oxide appear to induce cancers at multiple sites in some species.

The tumors associated with pesticide exposure in animals and humans are inconsistent. Some epidemiologic studies have associated lung, bladder, soft-tissue sarcomas, and lymphatic and hematopoietic cancers with organochlorine insecticides, whereas these chemicals have generally caused liver tumors in mice.

Cancer risks from non-occupational exposures to pesticides is of growing concern. Little data are available to adequately estimate the cancer burden for the general population. At the present time, however, the risks would appear to be smaller than from other carcinogens such as smoking, radon, and asbestos. This is because 1) excesses that have been observed in humans occur in occupational groups where exposures must have been much greater than those experienced by the general population; and 2) the excess for lung cancer (the most common cancer in the population) is small (less than 2-fold), and other cancers associated with pesticides are rare.

Pesticides have received considerable attention from experimentalists and epidemiologists for a number of years. Both make an important contribution to our understanding of this issue. The inconsistencies between experimental and epidemiologic results may point to a need for modifications in our current research strategy. First, many more pesticides have been evaluated for carcinogenicity in animal studies than in epidemiologic investigations. Numerous occupational groups with routine exposure can be identified. Most, however, have contact with many pesticides and multiple, concomitant exposures present a difficult problem. Pesticides, however, are targeted for specific pests; and, by conducting studies in a variety of situations, it should be possible to evaluate individual pesticides.

Experimental studies typically focus on a single pesticide, but given the pattern of concomitant exposure that occurs in humans, there is a need to utilize an experimental approach to reflect the human condition. Second, the paradigm of carcinogenicity for experimentalists and epidemiologists has been that carcinogens are electrophiles and mutagens; thus, pesticides would be active in early stages of carcinogenesis. Mechanistic investigations, however, have suggested a number of other ways in which carcinogens may act, including peroxisome proliferation and promotion. Both animal and human investigations should be designed to consider this potential.

As understanding of carcinogenesis has improved, there have been corresponding modifications in study designs. This is a continuing process, and the time may be ripe for additional modifications that can take into account the rapid advances occurring in the area of mechanisms of action. Promotional events, gap-junctional intracellular communication, and peroxisome proliferation may offer insight into the carcinogenic action of some pesticides. Failure to make such modifications may result in false negatives or animal positives whose relevance to humans is uncertain; either circumstance would severely limit our ability to make sound scientific and societal decisions.

There is a need in both experimental and epidemiologic investigations to develop the capability to evaluate effects at different stages of carcinogenesis. In epidemiologic studies, risks associated with exposure should be assessed relative to the timing of exposure without the prevailing view that only the earliest exposures are relevant. Experimental designs should include the capability of assessing promotional activity, in addition to the action of initiators.

RECOMMENDATIONS

The Cancer Working Group on Pesticides and Carcinogenesis makes the following recommendations:

1. Make all toxicologic data submitted to government agencies publicly available.

2. Determine mechanisms of action and pharmacokinetics of pesticides to understand the relevance to human risk, particularly for pesticides that have been associated with increased risk in epidemiologic studies and were not positive in animal testing.

3. Develop and validate methods to assess historical pesticide exposures that have occurred over the time span relevant for carcinogenesis.

4. Identify factors which predispose to increased cancer risks from pesticides.

Other recommendations of importance, but of lower priority than those above, are:

5. Apply epidemiologic study designs and analytical methods to identify and distinguish early stage carcinogens (i.e., initiators) from late stage carcinogens.

6. Most chronic animal pesticide studies have been ingestion studies, while the most important exposures for occupational groups are dermal and inhalation. More research on the relationships between the three exposure routes is needed.

7. Develop methods to evaluate the effects of intermittent and low level exposures to pesticides.

8. Develop toxicologic study methods to enable evaluation of co-carcinogens and promoters to mimic the human environment.

9. Critically re-evaluate the role of "maximum tolerated dose" and "no threshold" in the risk assessment of carcinogenicity of pesticides in humans.

10. Conduct epidemiologic research on populations other than mixers and applicators who are exposed to pesticides.

11. Conduct epidemiologic research on the possible carcinogenic effects of organophosphate pesticides, which have been rapidly replacing organochlorine pesticides.

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CHAPTER 5

IMMUNOLOGIC EFFECTS OF PESTICIDES

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SUMMARY

The immune system plays a critical role in resistance to infectious disease and growth of neoplastic tissue. This is demonstrated by the association between increased incidences of opportunistic infections, such as *Pneumocystis carinii*, and Kaposi's sarcoma in AIDS patients, or lymphomas in transplant recipients undergoing immunosuppressive therapy. There is concern that environmental or occupational exposure to pesticides may pose a health risk to humans through damage to the immune system. Evidence exists that pesticides can produce hypersensitivity reactions in humans. These examples originate from populations that have been inadvertently or occupationally exposed rather than from the population at large. In contrast, while ample evidence of pesticide-induced immunosuppression in experimental animals has accumulated, only limited examples of pesticides that cause immuno-suppression in humans have been found. This may be due, in part, to the lack of sensitivity of routinely used measurements of immune function and to the diversity of responses observed in those tests among exposed individuals. However, the lack of substantial human data as compared with the abundance of animal data describing pesticide-induced immunosuppression should not be interpreted as an absence of potential risk to the immune system of humans.

INTRODUCTION

The immune system is composed of a complex set of cellular and chemical components that protect the body against foreign antigens (e.g., bacteria, viruses, fungi, foreign tissue) while not responding adversely to "self." The distinction between "self" and "nonself" is made by a complex recognition system that depends on specific receptor molecules associated with the cell surface of T- and B-lymphocytes. Nonspecific effector mechanisms that amplify the specific T- and B-cell responses are also important in the immune response. These nonspecific features include mononuclear phagocytes, natural killer cells, polymorphonuclear leukocytes, and the complement system. Optimal functioning of the immune system requires that specific cells and cell products interact with each other in a sequential, regulated manner. Immunotoxicology is the study of the undesired biologic effects resulting from the interaction of foreign substances (xenobiotics) with the immune system. Toxic responses might arise when the immune system either: (1) serves as a passive target of chemical insult, leading to altered function (i.e., immunoenhancement, immunodeficiency); or (2) responds to chemicals or chemically modified "nonself" proteins (antigens) as part of a specific immune response, resulting in hypersensitivity, allergy or autoimmunity.

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Methods for evaluating potential injury to the immune system in experimental animals when it serves as a passive target for insult can be categorized. These methods are generally used in conjunction with a subchronic exposure regimen to the test compound of interest. One group of more commonly performed methods focuses on evaluating pathologic, hematologic, and anatomic parameters relevant to the immune system. These include body and lymphoid organ weights, histopathology and cellularity of lymphoid organs, as well as white blood cell counts and differentials. A second group of methods evaluates immune cell function either by quantifying adaptive immune function or evaluating host resistance to infectious or tumor challenge following chemical exposure. The functional assays are sensitive and especially useful for identifying specific defects among immune cell populations and assessing immune reserve. The host resistance models provide a holistic evaluation of immune function and put into perspective the clinical relevance of changes in immune function, thus aiding in risk assessment.

Systematic approaches for immunotoxicity assessment have been proposed. One such approach advocates a tiered testing scheme in rodents (Luster et al., 1988). The first tier of assays initially identifies immunomodulatory compounds. The second tier provides added perspective as to the biological significance of effects observed in the first tier, as well as data useful in elucidating mechanisms of action. An example of such an approach is shown in Table 5-1. The advantage to this approach is that a great deal is known about the structure and function of the rodent immune system, since it has been studied as a model for humans. This tier system is designed to evaluate chemically induced immunotoxicity in normal, healthy, young adult animals. In many cases, immune function tests similar to those described in Table 5-1 for rodents are used in the clinic for humans. There are, however, some limitations with these tests as applied to humans. One

TABLE 5-1. Panel For Detecting Immune Alterations
Following Pesticide Exposure in Rodents[a]

Parameter	Procedures	
	Screening (Tier I)	
Immunopathology	Hematology--complete blood count and differential; weights--body, spleen, thymus, kidney, liver; cellularity--spleen; histology--spleen, thymus, lymph node	
Humoral-mediated immunity	Enumerate IgM antibody plaque-forming cells to T-dependent antigen sheep red blood cells (SRBC); LPS mitogen response	
Cell-mediated immunity	Lymphocyte blastogenesis to mitogens (Con A) and mixed leukocyte response (MLR) against allogeneic leukocytes	
Nonspecific immunity	Natural killer (NK) cell activity	
	Comprehensive (Tier II)	
Immunopathology	Quantitation of splenic B-and T-cell numbers	
Humoral-mediated immunity	Enumeration of IgG antibody response to SRBCs	
Cell-mediated immunity	Cytotoxic T lymphocyte (CTL) cytolysis; delayed hypersensitivity response (DHR)	
Nonspecific immunity	Macrophage function-quantitation of resident peritoneal cells, and phagocytic ability (basal and lymphokine activated)	
Host resistance challenge models	Syngeneic tumor cells:	• PYB6 sarcoma • B16F10 melanoma
	Bacterial models:	• *Listeria monocytogenes* • Streptococcus species
	Viral models:	• Influenza
	Parasite models:	• *Plasmodium yoelii*

[a] The testing panel was developed using B6C3F1 female mice.
(Adapted from Luster et al., 1988)

such limitation is the inability to manipulate experimentally the human immune system as freely as is done in rodents. Such useful assays as measurement of the specific antibody plaque-forming cell response or host challenge with an infectious agent or tumor are not possible in humans. Furthermore, there is greater variation in responses in humans relative to that seen in hybrid or inbred rodents. Nevertheless, the tiered approach provides a logical, cost-effective means of assessing immune function useful for preclinical safety assessment of pesticides.

Methods for evaluating the immune system's response to chemicals or chemically modified "nonself" antigens are also used for safety assessment. There are a number of guinea pig test methods for predicting contact allergy in humans (Andersen and Maibach, 1985). These are summarized in Table 5-2. The guinea pig maximization test (GPMT) (Magnusson and Kligman, 1969) is often used to provide an estimation of inherent sensitization potential. Although it is most sensitive in that regard, it bypasses exposure considerations and can overestimate the sensitization potential of weak/moderate sensitizers (Andersen and Hamann, 1984; Andersen and Maibach, 1985; Maguire and Cipriano, 1984). Nonadjuvant topical guinea pig skin sensitization (GPSS) test methods, on the other hand, may be less sensitive, but they tend to avoid the false positive results of adjuvant methods (Bronaugh and Maibach, 1984) and may provide better perspective for human exposure. Human predictive data from the repeat insult patch test (HRIPT) and actual use test data can also be valuable in assessing sensitization risk (Robinson et al., 1989). For major chemical components, HRIPT testing should be preceded by appropriate GPSS testing to minimize the risk of sensitization in the test subjects. The occluded patch test (Buehler test) in guinea pigs is a useful method for primary assessment of skin sensitization potential in such cases (Buehler, 1985). It provides an exaggerated topical exposure through occlusion of the skin and readily detects strong and moderate, as well as many weak, human sensitizers. Properly conducted, it appears to provide a sufficient estimation of sensitization potential prior to human exposure, while avoiding overestimation of skin sensitization risk (Robinson et al., 1990). Several human patch test methods are also utilized for predictive skin sensitization testing of chemicals or formulations (Stotts, 1980). Although not required by regulatory agencies, data from such tests are valuable in assessing sensitization risk to humans (Robinson et al, 1989).

POTENTIAL FOR ADVERSE EFFECTS OF PESTICIDES ON THE IMMUNE SYSTEM

Exposure to pesticides can result in a variety of interactions with components of the immune system. These interactions range from modulation

TABLE 5-2. Guinea Pig Skin Sensitization Test Methods

Test	Route of Induction Exposure	Freunds' Complete Adjuvant	Occlusion	Route of Challenge Exposure
Draize	ID[a]	-	-	ID
Buehler	EC[b]	-	+	EC (occluded)
Ear flank	EC	-	-	EC (open)
Open epicutaneous test	EC	-	-	EC (open)
Guinea pig maximization test	ID + EC	+	+	EC (occluded)
Split adjuvant test	EC	+	+	EC (occluded)
Single injection adjuvant test	ID	+	-	EC (occluded)
Optimization test	ID	+	-	ID + EC (occluded)
TINA[c]	IM[d] + ID + EC	+	+	EC (occluded)

[a] Intradermal.
[b] Epicutaneous.
[c] Tierexperimenteller Nachweistest.
[d] Intramuscular.

of functional immune responses to development of hypersensitivity. The large number of published reviews on these subjects in recent years underscores the continued interest and concern for potential health effects of these compounds (Caspritz and Hadden, 1987; Cronin, 1980; Dean and Lauer, 1984; Edmiston and Maddy, 1987; Exon et al.; 1987; Luster et al., 1987; Sharma, 1988; Street, 1981). The following sections summarize data concerning pesticide-induced immune dysfunction and hypersensitivity.

Immune Dysfunction

An association between suppression of immune responsiveness and increased incidence of infection and neoplastic disease is clear. This association is perhaps best demonstrated clinically in organ transplantation or cancer patients receiving immunosuppressive drugs to prevent rejection or to control neoplastic growth (Allen, 1976; Penn, 1985). This association is also illustrated in the Acquired Immune Deficiency Syndrome (AIDS), where functional loss of immune responsiveness following infection with the human immunodeficiency virus (HIV) is associated with infection with *Pneumocystis carinii* and other opportunistic pathogens and development of a rare form of cancer known as Kaposi's sarcoma (Gottlieb et al., 1981).

A large body of evidence has also accumulated over the past 20 years that exposure to environmental chemicals, many of which are pesticides or contaminants of pesticide formulations, can produce immune dysfunction (i.e., altered immune function and altered host resistance) in experimental animals following acute and subchronic exposure. Some of these data are summarized in Table 5-3. Since certain classes of pesticides (e.g., organochlorine compounds) are stable and are capable of persisting in the environment and crossing the placental barrier, they may represent a particularly important group of compounds from an immunotoxicologic standpoint in terms of chronic exposure to the adult and neonate.

In addition to the active chemical(s), pesticide formulations also contain so-called inert ingredients and, occasionally, unwanted by-products of the manufacturing process. The biologic significance of exposure to these additional components is of increasing importance. For example, technical grade pentachlorophenol (T-PCP) suppresses the immune response of laboratory animals, whereas analytical grade PCP does not (Kerkvliet et al., 1982a,b). The immunosuppressive effects of T-PCP were subsequently shown to be mediated by immunotoxic chlorinated dibenzo-*p*-dioxin and dibenzofuran contaminants (Kerkvliet et al., 1985). Similarly, formulations of 2,4,5-trichlorophenoxyacetic acid (a component of Agent Orange) manufactured between 1958 and 1969 were contaminated with the highly immunotoxic 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) at levels ranging from 1-32 ppm

TABLE 5-3. Examples of Pesticides Reported To Modulate Immunity in Experimental Animals and Humans

Pesticide	Species	Summary of Effects	Reference
Organophosphates			
Methylparathion	Rabbit	Thymus atrophy and reduced DTH response	Street and Sharma, 1975
	Mouse	Decreased host resistance to *Salmonella typhimurium* infection	Fan et al., 1978 Street, 1981
Parathion	Mouse	Altered colony forming activities of bone marrow hematopoietic stem cells	Gallicchio et al., 1987
Malathion	Mouse	Suppression of CTL response *in vitro*	Rodgers et al., 1985
Organochlorines			
DDT	Rabbit	Thymus atrophy and reduced DTH response	Street and Sharma, 1975
Mirex	Chicken	Decreased IgG levels	Rao and Glick, 1977
Hexachlorobenzene	Mouse	Increased sensitivity to endotoxin and malaria challenge	Loose et al., 1978
	Rat	Increased humoral immune responses to tetanus toxoid and delayed-type hypersensitivity to ovalbumin	Vos et al., 1983

TABLE 5-3. Examples of Pesticides Reported To Modulate Immunity in Experimental Animals and Humans (Continued)

Pesticide	Species	Summary of Effects	Reference
Dieldrin	Mouse	Decreased AFC response and increased susceptibility to viral infection	Bernier et al., 1987 Krzystyniak et al., 1985
Chlordane	Mouse	Decreased contact hypersensitivity after *in utero* exposure	Spyker-Cranmer et al., 1982 Barnett et al., 1985a
	Mouse	Suppression of AFC responses and T-cell activity in a MLC reaction following *in vitro* exposure	Johnson et al., 1987
Pentachlorophenol	Mouse	Decreased host resistance to virus-induced tumor metastases	Kerkvliet et al., 1982a
Chlorophenoxy Compounds			
2,4-Dichlorophenoxyacetic acid (2,4-D)	Mouse	Enhanced T- and B-cell immune responses following dermal application	**Blakely and Schiefer, 1986**

TABLE 5-3. Examples of Pesticides Reported To Modulate Immunity in Experimental Animals and Humans (Continued)

Pesticide	Species	Summary of Effects	Reference
Carbamates			
Carbofuran	Rabbit	Reduced DTH response	Street and Sharma, 1975
	Mouse	Decreased host resistance to *S.typhimurium* infection	Fan et al., 1978
Aldicarb	Mouse	Decreased AFC response to sheep erythrocytes	Olson et al., 1987
	Human	Increased response to Candida antigen, increased number of lymphocytes expressing CD8 marker and decreased $CD4^+$/$CD8^+$ cell ratio	Fiore et al., 1986
	Mouse	No alterations in AFC response, B- or T-lymphocyte mitogenesis, host resistance to influenza virus infection, CTL response or percentages of T-cells, T-cell subpopulations or B-cells	Thomas et al., 1987, 1990

TABLE 5-3. Examples of Pesticides Reported To Modulate Immunity in Experimental Animals and Humans (Continued)

Pesticide	Species	Summary of Effects	Reference
Organotin Compounds			
Triphenyltin hydroxide (TPTH)	Rat	Reduced DTH response to tuberculin	Vos et al., 1984a
Tributyltin oxide (TBTO)	Rat	Reduced T cell-mediated, natural killer cell, and macrophage responses and decreased host resistance to *Trichinella spiralis* infection	Vos et al., 1984b

(Young et al., 1978, 1983). Recently, *O,O,S*-trimethylphos-phorothioate, a contaminant of malathion, has also been shown to alter immune function (Devens et al., 1985; Rodgers et al., 1986, 1987.) Thus, evaluations of immunotoxicity data from studies of commercially available pesticide formulations should consider the active and inert ingredients, as well as possible impurities.

The potential toxicity of pesticides to the developing immune system has received almost no attention (reviewed by Thomas and Faith, 1985). Studies with TCDD in rodents (Faith and Moore, 1977; Thomas and Hinsdill, 1979) showed more profound immunotoxic effects following perinatal exposure than after adult exposure, suggesting an increased sensitivity of the developing immune system to toxic damage than that of the adult. In recent studies, mice exposed to chlordane *in utero* displayed a decreased contact sensitivity response and an increased survival and antibody response to influenza as adults (Barnett et al., 1985a,b; Menna et al., 1985; Spyker-Cranmer et al., 1982). Exposure of mice to this compound as adults, however, did not alter cellular and humoral immune function (Johnson et al., 1986). Acute ten-day intermittent oral dosing of preweanling rats with 5 mg tributyltin oxide per kg body weight caused reversible immune alterations at a dose below the 10 mg/kg that altered immune parameters in adult rats (Smialowicz et al., 1988).

Despite sufficient evidence that pesticides affect functional immunity in rodents, difficulty arises in relating these changes to health risk in humans. In a study reported by Kundiev et al. (1986), even though hematologic changes were observed in humans where high concentrations of pesticide were present, no indication of altered immune status was found. In a study by Kashyap (1986), where immune status was altered, no change occurred in host resistance to disease. Specifically, no increase in infections were noted in workers occupationally exposed to four pesticides (malathion, parathion, DDT, and hexachlorocyclohexane) even though 73% of those exposed presented with altered levels of serum immunoglobulin. Increases in serum IgG and decreases in serum IgM and C-3 component of complement were reported in 51 men exposed to chlorinated pesticides when compared to a 28-man control group (Wysocki et al., 1985). However, a direct association between increased incidence of infection and change in immune status was absent. On the other hand, impairment of neutrophil chemotaxis accompanied by an increase in upper respiratory tract infection was correlated with length of occupational exposure in 85 workers handling organophosphate pesticides (Hermanowicz and Kossman, 1984).

The potential of aldicarb, a carbamate pesticide, to alter immunologic status has recently been studied in mice and humans. Olson et al. (1987) reported an unusual inverse dose-related suppression of the antibody response following exposure of mice to levels of aldicarb as low as 1 ppb for 34 days in drinking water. However, Thomas et al. (1987, 1990), using a similar exposure regimen, a similar mouse strain, and a more comprehensive testing configuration, observed no aldicarb-related effects on immunity, including susceptibility to infection. Another study by Fiore et al. (1986) suggested that women chronically ingesting low levels of aldicarb-contaminated groundwater had altered numbers of T-cells, including a decreased CD4/CD8 cell ratio. Confirmation of these results and interpretation of their biologic significance awaits further studies.

In 1984, an exothermic reaction occurred in a methyl isocyanate (MIC) storage tank at a pesticide production facility in Bhopal, India. MIC is used as an intermediate in the production of carbamate pesticides. A variety of effects on immune responses were reported in exposed individuals, including an increase in the number of T-helper and total T-cells but decreases in lymphocyte mitogenesis (Deo et al., 1987). Studies in mice showed that only minimal, if any, changes in immune function occurred following MIC exposure. Furthermore, these changes were thought to be a secondary consequence of respiratory toxicity (Luster et al., 1986). These results, coupled with normal responses observed in other tests, provided no clear pattern for immune dysfunction associated with MIC exposure. In summary, although there is ample and convincing evidence showing that pesticides or pesticide contaminants modulate the immune response of experimental animals, these changes cannot always be related to potential health risks in humans.

Induction of Hypersensitivity

In addition to inducing immune dysfunction, pesticides have the potential for immunotoxicity by modification of endogenous proteins or cells. These changes potentially could result in hypersensitivity or autoimmune reactions. With respect to the former, there are two types of responses reflecting distinct mechanistic arms of the immune system. Delayed-type (contact) hypersensitivity is a T-cell mediated inflammatory response, the cutaneous clinical manifestation of which is allergic contact dermatitis. Immediate hypersensitivity is an antibody-mediated response (IgE in humans) with such clinical manifestations as allergic rhinitis, asthma, and, in rare instances, systemic anaphylaxis. Both hypersensitivity responses require initial exposure to an allergen to induce the immune response. A subsequent (challenge) exposure elicits the clinical symptoms.

Both types of hypersensitivity responses have considerable impact on occupational health and safety. Contact hypersensitivity is not life-threatening, but has considerable economic and occupational disease importance, since it is responsible for a significant percentage of lost work hours. Immediate hypersensitivity can be life-threatening in cases of severe asthma or anaphylaxis, and exposure to the offending allergen must be avoided. The role that pesticides play in these types of allergic hypersensitivity reactions is discussed below.

Contact Hypersensitivity Pesticides have been implicated as causal agents of contact dermatitis, (Cronin, 1980; Johnsson et al., 1983; Mathias et al., 1983; Meding, 1986; Thormann, 1982). Furthermore, several investigators have speculated that the actual occurrence of contact dermatitis in agricultural workers is greater than the reported incidence (Cronin, 1980; Hogan and Lane, 1985; Matsushita and Aoyama, 1980; Matsushita, et al., 1985). This discrepancy may be due to the isolated nature of the work, movement from field to field, exposure to numerous chemicals, and lack of diagnostic follow-up. Given the widespread use of these chemicals, the incidence of confirmed pesticide-related contact dermatitis is rare. A published summary of over 1,900 occupational illness and injury reports related to pesticides listed 1,065 incidents of illness or injury due to pesticide exposure, of which 342 were skin-related complaints (Edmiston and Maddy, 1987). These 342 cases were out of an agricultural and pesticide applicator work force of approximately 800,000, which translates to only 0.43 cases per 1,000 workers per year. The actual incidence of contact dermatitis may be even lower, since the 342 cases included all skin illness and injury complaints (e.g., irritation, allergy, burns). Winter and Kurtz (1985) evaluated various environmental factors influencing the incidence of skin rashes in California vineyard workers. They reported the incidence of skin rash to be 24 per 1,000 workers per year, a figure that was considerably higher than the reported incidences for the general (2.1 per 1,000) or agricultural work force (8.6 per 1,000). In spite of this high incidence, little correlation could be made with any pesticide exposure. Most skin rashes were associated with exposure to high temperatures during thinning and harvesting operations. All occupational dermatitis in the agricultural work force, therefore, can scarcely be attributed to pesticides, since many other causal factors may be involved.

In instances where both animal and human sensitization test data exist, the results do not always agree (Table 5-4). Sensitization data for barban, a carbamate pesticide, provide an example where patch test results in animals and humans and occupational incidence of allergic contact sensitivity do not agree. Only two contact dermatitis case reports for barban appear in the literature (Brancacia and Chamales, 1977; Hogan and Lane, 1985) suggesting

TABLE 5-4. Comparison of Animal and Human Pesticide Sensitization Data

		Guinea Pig Skin Sensitization					Human Diagnostic Patch Test			
Material	Test	[Ind[a]], %	[Chall[b]], %	% Pos	Grade	Reference	No. of Subjects	[Chall], %	Result	Reference
DNCB	Buehler	0.05	0.05	100	N/A[c]	Buehler, 1985	2	0.1	+ + +	Adams et al., 1971
		0.01	0.01	40	N/A	Buehler, 1985		0.025	+ + +	
								0.0001	+ +	
	GPMT[d]	0.01 ID[e], 1 EC	0.01 EC[f]	100	Extreme	Wahlberg & Boman, 1985				
		0.01 ID, 1 EC	0.01 EC	75	Strong	Wahlberg & Boman, 1985				
Barban	Buehler	8	8	100	N/A	Hogan & Lane, 1985	2	0.1	+ + +	Hogan & Lane, 1985
								0.001	+ +/+ + +	
								0.0001	+ +	
								0.00001	+ +	
							1	10 formula	+ +	Brancacia & Chamales, 1977
Benomyl	GPMT	5 ID, 25 EC	2	100	Extreme	Matsushita & Aoyama, 1981	3	1	+/+ +/+ + +[g]	Van Joost et al., 1983
			0.2	100	Extreme		1	1	Pos[h]	Van Ketel, 1976
							4	10	+ +	Savitt, 1972

TABLE 5-4. Comparison of Animal and Human Pesticide Sensitization Data (Continued)

		Guinea Pig Skin Sensitization					Human Diagnostic Patch Test			
Material	Test	[Ind[a]], %	[Chall[b]], %	% Pos	Grade	Reference	No. of Subjects	[Chall], %	Result	Reference
Maneb	GPMT	5 ID, 25 EC	2	100	Extreme	Matsushita & Aoyama, 1980	3	5	+ + > + + + +	Nater et al., 1979
			0.5	100	Extreme			2	+ + > + + + +	
			0.2	100	Extreme			1	+ > + + +	
			0.05	100	Extreme		1	1	Pos	Adams & Manchester, 1982
Naled	GPMT	5 ID, 25 EC	2	90	Extreme	Matsushita et al., 1985	3	60	Pos	Edmundson & Davies, 1967
			0.2	0	Weak					
Malathion	GPMT	10 ID, 10 EC	20	54	Moderate	Magnusson & Kligman, 1969				
		5 ID, 25 EC	2	70	Strong	Matsushita et al., 1985	200	1	3% Pos	Milby & Epstein, 1964
			0.2	10	Mild					
Captan	GPMT	5 ID, 25 EC	1	83	Extreme	Marzulli & Maguire, 1982	509	1	3% Pos	Cronin, 1980
							200	1	5.5% Pos	Lisi et al., 1986
							200	0.5	4% Pos	Lisi et al., 1986

TABLE 5-4. Comparison of Animal and Human Pesticide Sensitization Data (Continued)

		Guinea Pig Skin Sensitization					Human Diagnostic Patch Test			
Material	Test	[Ind[a]], %	[Chall[b]], %	% Pos	Grade	Reference	No. of Subjects	[Chall], %	Result	Reference
Sodium chlorate	GPMT	5 ID, 25 EC	5 2	80 30	Strong Moderate	Matsushita et al., 1975a	38	10 1 0.1	5.3% Pos 10.5% Pos 10.5%	Matsushita et al., 1975b
Sodium dichloro-propionate	GPMT	5 ID, 25 EC	5 2	10 0	Mild Weak	Matsushita et al., 1975a	38	10 1 0.1	13.2% Pos 10.5% Pos 13.2% Pos	Matsushita et al., 1975b

[a] Induction concentration.
[b] Challenge concentration.
[c] Not applicable.
[d] Guinea pig maximization test.
[e] Intradermal.
[f] Epicutaneous.
[g] Severity of diagnostic patch reaction.
[h] Positive.

that the human sensitization potential is weak. However, Hogan and Lane elicited positive reactions in diagnostic patch testing with barban at concentrations as low as 100 parts per billion and induced a primary sensitization response 10 days after a single patch exposure at a 1% concentration. They also reported a 100% incidence of positive guinea pigs by occluded patch testing at a concentration of 8% barban in acetone.

The pesticides malathion, captan, benomyl, maneb, and naled are strong to extreme sensitizers as measured by GPMT; however, human sensitization data do not always agree. For example, although human maximization test (HMT) data on technical grade malathion support its classification as a strong sensitizer (Kligman, 1966), such a rating was not confirmed by an International Contact Dermatitis Research Group survey of 455 individuals, in which one individual tested positive (Cronin, 1980). The lack of clinical reports of dermatitis from malathion use (including direct skin contact as a delousing agent) makes it apparent that the animal and human predictive tests (GPMT and HMT) that indicated strong to extreme sensitization potential may have overestimated the sensitization hazard. Furthermore, the offending chemical in technical grade malathion is diethyl fumarate (Fisher, 1986; Milby and Epstein, 1964); reduction of this constituent has further lowered the sensitization potential of this pesticide.

Captan was also classified as an extreme sensitizer by GPMT at a 1% to 5% concentration (Marzulli and Maguire, 1982). However, earlier HRIPT data showed only a 4.4% incidence of induced sensitization at 1% concentration (Marzulli and Maibach, 1973). Survey data using diagnostic patch testing at 0.5% to 1% concentration (Cronin, 1980; Lisi et al., 1986) tend to confirm the HRIPT results, but not the GPMT data. Diagnostic patch testing in isolated cases of occupational dermatitis has shown positive reactions to benomyl, maneb, and naled at patch test concentrations as low as 1% (Edmundson and Davies, 1967; Nater et al., 1979; Savitt, 1972; van Joost et al., 1983; van Ketel, 1976). However, GPMT data indicating strong/extreme sensitization potential would suggest a much greater incidence of dermatitis than that reported in the literature.

In the case of dinitrochlorobenzene (DNCB), the animal and human data tend to agree. Dose response sensitization reactions are seen in Buehler-type (occluded patch) GPSS testing (Buehler, 1985) with somewhat greater incidence of sensitization by the GPMT at similar concentrations (Wahlberg and Boman, 1985). Human studies show positive reactions below a 0.2% w/v DNCB in vehicle (acetone) after a single occluded patch induction exposure (Friedman et al., 1983). Diagnostic patch testing of two workers with clinical dermatitis thought to be due to DNCB exposure resulted in moderately positive patch test reactions at a concentration as low as 1 part per million (Adams et al., 1971). Therefore, the worker reactions and diagnostic patch test data from animal and human predictive testing support the classification of DNCB as a strong sensitizer and emphasize the importance of restricting industrial use of the material to closed systems to avoid spillage.

In summary, although pesticides have been implicated as causal agents of contact dermatitis in agricultural workers, the incidence of confirmed pesticide-related contact dermatitis is not common. The intermittent and migratory nature of the field work leading to infrequent diagnostic follow-up of employees with complaints may be one reason for this. Furthermore, the animal sensitization test data are apparently not always predictive of human contact sensitization risk.

Immediate Hypersensitivity Reports that pesticide exposure causes the development of immediate allergic reactions such as rhinitis, asthma, or anaphylaxis are difficult to confirm. Many patients with underlying allergic disease indicate symptom exacerbation after exposure to pesticides. However, such reactions may be "irritative" rather than "allergic" in nature. Some evidence indicates that certain individuals may develop asthma in response to pesticides. Isolated case reports (Bryant, 1985) showed that the asthmatic response to organophosphorous pesticides was not due to inhibition of acetylcholinesterase activity, since the cholinergic antagonist atropine failed to block the response. Although clinical characteristics of immediate hypersensitivity responses suggest IgE sensitization, it has not been established whether such sensitization occurs in humans. However, preliminary experiments in animals support the possibility of IgE sensitization to pesticide derivatives. For example, mice immunized with 2,4-dichlorophenoxyacetic acid or malathion conjugates demonstrated IgE antibody following intraperitoneal but not epicutaneous immunization (Chiorazzi et al., 1977; Cushman and Street, 1982, 1983).

From a clinical viewpoint, strict criteria to define a target organ response will enhance the likelihood that the true prevalence of pesticide-induced hypersensitivity can be elucidated. For example, following exposure to an aerosol pesticide formulation, seven individuals with a history of asthma complained of chest tightness (Newton and Breslin, 1983). However, only one subject demonstrated significant airway obstruction, as measured by a 35% fall in FEV_1. Although two others had obstructive changes in measures of small airway function and in maximum mid-expiratory flow, reductions in FEV_1 were not readily noted. In summary, these examples illustrate that more information is needed to determine the precise role of pesticides in the subsequent development of allergic diseases and asthma in humans.

Autoimmunity Autoimmune diseases are disorders of immunologic regulation in which many different factors (e.g., genetic, viral, hormonal, psychological, and environmental) may play a role. Autoimmune disease can belong to any of the four general classifications of hypersensitivity (Coombs and Gell, 1968) and include production of autoantibodies, destructive inflammatory cell infiltrates in various target organs, and deposition of immune complexes in vascular sites. Resulting diseases include rheumatoid arthritis, systemic lupus erythematosus, autoimmune thyroiditis, hemolytic anemias, and sclerodermas.

Reports that pesticides induce hematologic disorders resembling autoimmune disease are rare, and the data, when available, are difficult to interpret. For example, the presence of anti-dieldrin IgG antibody in serum and coating the red cell membrane formed the basis for a positive antiglobulin test in an individual with symptoms of immunohemolytic anemia (Hamilton et al., 1978). Furthermore, neutralization of serum antibody by prior incubation with the antigen (dieldrin) nullified the reaction. Because the anemia was cured following splenectomy suggested that the spleen played a central role in dieldrin storage, red cell destruction and immunopathology. On the other hand, general cellular toxicity, rather than an autoimmune process, following pesticide exposure may have been responsible for several reports of aplastic anemia in humans (Loge, 1965; Roberts, 1963; Sanchez-Medal et al., 1963). However, antibodies produced in experimental animals to conjugates of pesticides, such as DDT and malathion (Centeno et al., 1970), suggest that similar immunopathologic responses leading to autoimmunity could occur in man. Although current mammalian toxicology data requirements for pesticide registration should allow for the detection of autoimmune-related hematologic disorders and certain histologically identifiable organ-specific changes, other autoimmune responses may remain undetected. At the present time, laboratory animal model systems for autoimmune diseases are not well developed.

REGULATORY STATUS ON IMMUNOTOXICOLOGY OF PESTICIDES

Chemical Pesticides

Data derived from toxicology studies with laboratory test animals serve as a basis for the EPA to predict the likelihood of adverse health effects arising from exposure of humans to pesticides. Chemical pesticides undergo an extensive toxicologic evaluation prior to receiving EPA approval for registration and legal commercial use. Data requirements for chemical pesticides (EPA, 1984) include a battery of acute toxicity studies (i.e., oral, dermal, and inhalation) in the rat and subchronic and chronic feeding studies in two test animal species. Teratology, reproduction, mutagenicity, and metabolism studies are also required. Procedures for properly conducting these studies are provided in the EPA's Subdivision F guidelines. Although no studies are required to evaluate the immune system as a specific target for pesticide toxicity, a limited number of direct and indirect immune system parameters are evaluated. These parameters include a total and differential leukocyte count, total serum protein determination, hematology determinations, organ weights (although lymphoid tissues and organs are not specifically designated), and conventional histopathology. Pesticide-induced toxicity to even the most fundamental humoral- and cell-mediated immune responses could be overlooked by such limited data. Thus, the EPA is in the process of revising its Subdivision F guidelines to provide a more effective screen for pesticides as immunotoxicants (Sjoblad, 1988).

Naturally Occurring Pesticides

The EPA has designated certain naturally occurring pesticides that act by a specific mechanism in mitigating or controlling a target pest as "biochemical pest control agents" (BPCAs). This group of pesticides, considered distinct from conventional chemical pesticides, includes substances such as pheromones, plant and insect growth regulators, hormones, and enzymes. In general, the mammalian toxicology data requirements for BPCAs are less extensive than for conventional chemical pesticides, which is consistent with the belief that the biochemical pesticides would be less toxic because they are naturally occurring and act by a specific mode of action. Also, human exposure might be less, since these pesticides are often used at low application rates. The mammalian toxicology data requirements for registration of BPCAs are specified in 40 CFR 158.165 EPA, 1984, and protocols for proper conduct of these studies are detailed in Subdivision M guidelines, which were published in 1982. BPCAs are evaluated by using a tiered testing scheme. Mammalian toxicology studies required in Tier I include acute tests of oral, dermal, and inhalation toxicity, as well as mutagenicity and immune system testing. Hypersensitivity, teratogenicity, and subchronic exposure studies are required if human exposure to toxic levels is likely to occur under the proposed use conditions. If adverse effects are observed from the Tier I tests, additional mutagenicity and immunotoxicity tests are required in Tier II and chronic toxicity and oncogenicity studies are required in Tier III.

The EPA has revised the immune system study sections in Tier I and Tier II of the 1982 version of Subdivision M guidelines. These parts of the guidelines were upgraded to accommodate the rapid, recent progress made in the area of immunotoxicity testing of xenobiotics. The revised version for Tier I includes evaluations for spleen, thymus, and bone marrow cellularity;

humoral immune effects (immunoglobulin responses to antigen or antibody plaque-forming cell assay); and effects on nonspecific cell-mediated immunity (natural killer cell activity and macrophage function). If adverse effects are observed in these Tier I tests, additional Tier II studies would include, but not necessarily be limited to, an evaluation of effects of the BPCA on host resistance to infectious agents or to tumor cell challenge and an evaluation of the time for recovery from adverse effects (Sjoblad, 1988).

SPECIFIC CONCERNS WITH EXISTING STATE OF KNOWLEDGE OF THE CHRONIC EFFECTS OF PESTICIDES ON THE IMMUNE SYSTEM

The Immunotoxicology Working Group has identified four specific categories as critically important to improve the knowledge of the chronic effects of pesticides on human health.

Animal to Human Extrapolation

The existing data bases for humans and experimental animals provide some interspecies correlations of chemically induced immune perturbations. Good examples of these correlations exist in the literature associated with the development and use of the drugs cyclophosphamide and cyclosporin-A (Gershwin et al., 1974; Hess et al., 1988; Turk and Parker, 1979) and with certain halogenated aromatic hydrocarbons (reviewed by Trizio et al., 1988).

Two important criteria used for extrapolation from experimental animal models to man are valid for the immune system. The first criterion is the use of an animal model in which the pesticide has the same pharmacokinetic pattern as in man. Ideally, information on absorption, distribution, biotransformation, and excretion of the pesticide would be available in both species during pesticide research and development. A second criterion is the use of an experimental animal model in which the desired end point can best be measured. It is generally agreed that the immune system of the rodent and that of the nonhuman primate are similar to that of man. Furthermore, compounds known to modulate the immune system in man do so similarly in rodents. Although good animal models exist, *in vitro* follow-up studies may be useful in elucidating possible differences in sensitivity of chemically mediated immunotoxicity in laboratory animal models relative to humans. Peripheral blood leukocytes can be easily obtained from humans and rodents and used for a number of *in vitro* immunologic procedures. When intrinsic limitations of the *in vitro* systems are defined, comparison of results using cells from both species exposed to the compound *in vitro*, along with data generated using cells from rodents exposed *in vivo*, may provide additional insight into possible human immunotoxicologic effects of the compound as well as species differences in immune cell sensitivity. With regard to contact hypersensitivity, extrapolating to humans from the animal sensitization data presents some difficulty because, as reported above, the published animal test data (primarily GPMT results) generally indicate a sensitization potential greater than that reported for humans.

Epidemiology

The usefulness of epidemiology studies in linking environmental exposure to pesticides with altered immune status and subsequent changes in resistance to disease is limited (reviewed by Ellis, 1988). Variability among the population with respect to what is considered a "normal" immune response and the confounding factors involved in interpretation of this response decrease the probability of using broad-based epidemiologic studies to identify xenobiotics that are responsible for causing immune injury. Subtle perturbations in immune function following exposure to environmental chemicals may not, in every instance, result in a measurable health effect. Alternatively, subtle changes in certain immune functions could conceivably increase the likelihood of adverse immune-related health effects only during the brief period when the precipitating events (e.g., infectious challenge) are present. However, current epidemiologic evidence concerning some of the more toxic environmental chemicals does not provide support for a strong link between subtle immunologic perturbations and biologically relevant changes in resistance to disease. It cannot be ruled out, however, that problems associated with studies of this nature, such as the variability in immune responses associated with the human population, have precluded the detection of evidence for this link.

Contact hypersensitivity is known to account for a significant percentage of occupational adverse skin reactions and, although case reports are rare, is thought to be an important cause of skin reactions in pesticide-exposed workers (Cronin, 1980). However, the epidemiology must be improved to confirm the pesticide association since other causal factors may be involved (Winter and Kurtz, 1985).

Public Perception of Risk

Public perception of risk associated with pesticide exposure has become a significant factor in overall environmental risk management. It is one criterion used by those who are occupationally exposed to determine the level of care in handling the compounds. It is also a major consideration of policy makers. Unfortunately, public perception of risk can be influenced by the manner in which scientifically valid and objective studies are misinterpreted or incompletely reported.

Regarding the immune system as a target for pesticides, the public holds two perceived concerns. One concern involves hypersensitivity responses to pesticides. As previously discussed, data in both experimental animals and humans demonstrate that exposure to xenobiotics, including pesticides, can result in sensitization and subsequent hypersensitivity responses. These responses can at times be debilitating and, in the case of anaphylaxis, life-threatening. The second concern deals with potential xenobiotic-induced suppression of the immune system. As a result of the AIDS pandemic, the public has become aware of the role of the immune system in host defense and recognizes the potential problems associated with a compromised immune system. Considering the limited scientific evidence of the ability of low-level environmental exposure to xenobiotics to significantly alter the immune system, the present observations should be interpreted with caution and the relevance to humans placed in its proper perspective. Many of these exposures can alter immunologic measurements in experimental animals and sometimes in humans at relatively high doses. The inability to, as yet, associate pesticide exposure definitively with adverse health effects of immunosuppression in humans may be the result of our inexperience in identifying the consequences of minor immunologic perturbations, insufficient exposure levels, the difficulties encountered in defining "normal" immune status in humans, and the kinetics involved in immune modulation and the subsequent appearance of disease.

Public concern for possible chemically induced immune disturbances in humans has grown, in part, from recent studies suggesting T-cell abnormalities in women following consumption of aldicarb-contaminated groundwater (Fiore et al., 1986), as well as media attention given to the potential health effects of individuals exposed to Agent Orange. This concern has been fueled by statements of an immunologic etiology for a recently described disease sometimes referred to as "environmental illness," as well as court litigations ascribing immunologic changes associated with exposure to environmental contaminants including pesticides (Elam v. Alcolac). Little scientific evidence exists to support these suggestions or to support the idea that environmental exposure to xenobiotics, in general, has influenced the immunocompetence of the general population at large.

Exposure Variables

As with other target organs, the kinetics of immune system toxicity and recovery from these effects are important considerations when designing studies or interpreting data to assess potential human risk. The toxic effects of pesticides on the immune system, as in other organ systems, are a function of dose; thus, levels can be established which should allow decisions to be made with respect to benefits and risk. The duration of chemical exposure for *in vivo* studies is another important consideration. An exposure time shorter than 90 days may be justified because of the fairly rapid turnover of the immunocompetent cells. If experimental immunization is incorporated into the study design, timing of challenge and measurement of response relative to pesticide exposure are critical variables that must also be considered. The immune response is particularly sensitive during proliferation and differentiation events; whereas, resting lymphocytes are resistant to damage. The immune system is particularly vulnerable to damage during the perinatal period. Thus, an exposure that maximizes pesticide levels in the lymphoid tissue at this critical time and allows for steady-state levels to occur should maximize the effect on the immune system. These toxicokinetic factors and critical temporal relationships must be considered when interpreting animal and human pesticide immunotoxicity data, extrapolating animal immunotoxicity data to humans, and formulating testing recommendations.

CONCLUSIONS

1. **With the exception of occupational exposure to pesticides leading to contact hypersensitivity, the data are insufficient to conclude that chronic exposure to pesticides at environmentally relevant levels results in interactions with the immune system which adversely influence human health.**

The discipline of immunotoxicology represents a relatively new area in toxicology. As a result, only a limited data base exists in which pesticides have been adequately examined in laboratory and epidemiology studies. Studies conducted in rodents, however, suggest that selected pesticides or their by-products can adversely affect the immune system through disruption of cell maturation, regulation, or cytotoxic processes and lead to altered host resistance and disease. With the exception of certain chemicals (e.g., halogenated aromatic hydrocarbons), no substantial evidence yet exists that exposure to pesticides, either in the workplace or through casual contact, induces significant immune dysfunction in humans leading to disease. This may be due to any one or more of the following:

- Exposure levels required to induce immune change in animals may not be achieved in humans.

- Based upon the effects observed in rodents, the limited tests currently routinely performed in humans may not detect immune changes if they do exist.

- Adverse health effects associated with subclinical immune dysfunction in humans are difficult to quantitate (e.g., increased incidence and/or severity of common infections).

- Diseases associated with immune dysfunction can become detectable only after a long latency or following more severe immunomodu-lation and, as a result, may be difficult to initially detect.

- Although the rodent and human immune systems are similar, subtle differences in detoxification mechanisms and adaptation processes may render humans more resistant to pesticide-induced immunologic damage.

- The "normal" immune response in humans is quite heterogeneous, making it difficult to discern a subtle adverse effect without a large experimental cohort.

2. Experimental and clinical data demonstrate that certain pesticides (e.g., chloronitrobenzenes, carbamates, captans, and organophosphorus compounds) induce contact hypersensitivity responses in experimental animals and humans.

Animal test results (primarily guinea pig maximization test data) tend to exaggerate the sensitization potential of most pesticides tested relative to human exposure. However, in humans, the true incidence of pesticide-related contact dermatitis is likely somewhat greater than the reported incidence due to incomplete tracking (i.e., diagnosis or epidemiology) of adverse skin reactions associated with occupational pesticide exposure.

3. Although the immune system may have unique sensitivities for detecting pesticide-induced toxicity, it is similar to other organ systems in that dose-response relationships exist and threshold values for toxicity can be demonstrated.

Safety factors based upon adverse immunologic effects of xenobiotics, including pesticides, can be developed using currently available methodologies. The uncertainties associated with these safety factors are no less meaningful than those associated with other toxicities. However, the major confounding

factor is the definition of "adverse immunologic effect." Consensus on this is currently being debated.

4. **Case reports give ample evidence that asthmatic-type reactions can be triggered by pesticide exposure. However, it has not been determined whether these reactions are strictly immunologic in nature.**

Immunization studies in animals to pesticide-protein conjugates have resulted in production of pesticide-specific allergic antibodies, suggesting that antibody-mediated adverse reactions can occur in humans. However, to date, there is no evidence for pesticide-induced IgE antibody responses in humans. Individuals with underlying allergy (atopics or asthmatics) may show increased symptomatology upon exposure to pesticides, but the mechanism at present is unknown.

5. **As to immune damage caused by pesticides, certain selected populations may be at a greater risk than the general public.**

These groups include pesticide manufacturers, pesticide applicators, residents of neighborhoods proximal to intensive pesticide use areas, atopic/asthmatic individuals predisposed to developing hypersensitivity, genetically predisposed individuals, patients on drug therapy, immunologically compromised individuals, and the young and aged.

RECOMMENDATIONS

1. Develop a more complete scientific understanding of the mechanisms of pesticide immunotoxicity in order to improve the data base for risk assessment.

2. Improve educational, diagnostic training, and follow-up procedures to determine the extent of pesticide-related contact dermatitis in occupationally exposed individuals.

3. Develop a more complete data base for defining the range of "normal" human immune function for use in well-designed epidemiology studies.

4. Identify and evaluate immune status of suspected "at-risk" groups within the general population that, for a number of reasons, are more likely to suffer immunologically related adverse health effects from chronic pesticide exposure.

5. There is no evidence that chronic exposure to low (subthreshold) concentrations of contact allergens induces contact sensitization. However, additional research should be directed toward better evaluation of this question, perhaps by conducting carefully controlled diagnostic patch test surveys.

6. Improve public perception of risk from pesticide exposure through educational mechanisms involving the mass media.

7. Improve the availability of data, both published and proprietary, to permit realistic assessment of adverse health effects from pesticides.

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CHAPTER 6

REPRODUCTIVE EFFECTS OF PESTICIDES

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INTRODUCTION

Over the past decade there has been growing concern about human reproductive vulnerability to disruption by xenobiotic substances, including pesticides. For example, the cover story of an issue of *U.S. News and World Report* (October 5, 1987) focused on infertility in the United States and suggested that some portion of reproductive disease was a result of environmental contamination.

This interest follows the publication of several volumes devoted to various aspects of reproductive toxicity. These include the proceedings of a conference sponsored by the World Health Organization in Italy (Vouk and Sheehan, 1982), a meeting sponsored by the Institute of Occupational Medicine and Hygiene in Helsinki (Hemminki, 1985), and a report on occupational health hazards in the workplace by the Office of Technology Assessment (OTA, 1985). Additional recent publications include an excellent annotated review by Barlow and Sullivan (1982) on the effects of selected xenobiotics on reproduction and several books outlining the principles of reproductive toxicology (Mattison, 1983; Dixon, 1985). These and other publications provide an index of the growing effort in reproductive toxicology by biologists, toxicologists, pharmacologists, epidemiologists, and other health scientists.

Two bibliographies have explored available literature on reproductive toxicology: the critical review of 48 industrial chemicals by Barlow and Sullivan (1982) and the bibliography by Pruett and Winslow (1982) prepared from a computer search of several data bases. The annotated bibliography compiled by Pruett and Winslow (1982) is a useful introduction to the clinical

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manifestations of human reproductive toxicology. The bibliography contains approximately 270 references published between 1963 and 1981. The bibliography categorizes reports by site of reported adverse effect in both the male and female. Slightly less than half the references deal with male reproduction and slightly more than half with female reproduction. The bibliography prepared by Barlow and Sullivan (1982) is a more critical assessment of the effects of 48 chemicals on reproduction. What is needed, however, is a much more extensive analysis of existing data on reproductive effects of xenobiotic substances, especially pesticides.

Barlow and Sullivan (1982) collected animal and human data on 48 chemicals of industrial interest (Table 6-1). All 48 chemicals had relevant pharmacology and toxicology data: 38 were positive, producing adverse effects in experimental animals, and 10 were negative. In no case were the data from experimental animal studies insufficient for assigning the chemical to a group.

TABLE 6-1. Reproductive Hazards of Industrial Chemicals

	P & T[a] Effects	E & G[b] Effects		Fertility		Pregnancy
		M	F	M	F	
ANIMAL						
Positive	38	11	11	9	9	17
Negative	10	4	2	6	5	9
No data	0	33	35	33	34	22
HUMAN						
Positive	19	8	6	2	1	6
Negative	0	0	3	1	1	1
No data	29	40	39	45	46	41

[a] Pharmacologic and toxicologic
[b] Endocrine and gonadal
[c] Positive = Studies performed suggest toxicity
Negative = Studies performed suggest no toxicity
No Data = No studies or inconclusive results

In humans, there were sufficient pharmacologic and toxicologic data on only 19 of the 48 chemicals, all positive. The remaining 29 had insufficient data for defining human toxicity. The authors then evaluated the available data from animals and humans addressing reproductive and developmental toxicity, endocrine or gonadal effects, alteration in fertility, and effects on pregnancy.

Data on 11 of the 48 chemicals suggested adverse endocrine or gonadal effects in male or female experimental animals; 4 were clearly negative in males and 2 in females. Most of the chemicals, however, had insufficient data to define gonadal toxicity in experimental animals or in humans. Effects of xenobiotic compounds on fertility were also poorly characterized, with no animal data on 33 or 34 compounds and insufficient human data on 45 or 46 chemicals. Although there is slightly more information on the effects of these 48 chemicals on pregnancy in experimental animals, human data are clearly lacking. This suggests two factors; for many chemicals, including many pesticides, animal data necessary to define reproductive or developmental hazard are not available, and where animal data are available, even minimal human data are lacking. Therefore, once a reproductive or developmental hazard has been identified, extrapolation across species will be necessary to define human risk.

Ultimately, the concern for reproductive vulnerability translates through the assessment of reproductive risk into regulations, such as California's Proposition 65, controlling human exposure to xenobiotic compounds. At the present time, reproductive risk assessment is accomplished by one of two processes: (1) application of a safety factor to the lowest dose that produces no reproductive effect; or (2) statistical modeling of the dose-response curve to reach some acceptable level of excess population risk for the reproductive effect. Reproductive risk assessment using these techniques will carry considerable uncertainty until broader data bases are available and better methods for characterizing the site and mechanism of action of reproductive toxicants across species is achieved.

Mechanisms of Action of Reproductive Toxicants

Reproductive toxicants may produce an adverse effect by one of several mechanisms. Some xenobiotic substances are direct-acting reproductive toxicants, either by virtue of chemical reactivity (e.g., germ cell destruction by ionizing radiation) or by structural similarity to endogenous molecules (e.g., hormone agonists or agonists such as phytoestrogens). Other xenobiotics interrupt reproduction indirectly either by metabolism to a direct-acting toxicant (e.g., hormone or reactive intermediates) or by endocrine alterations (e.g., increased steroid clearance). Selected examples of pesticides producing reproductive toxicity by one of more of these mechanisms will be reviewed in this chapter.

Species differences in reproductive toxicology can be complex and dependent on mechanisms of hormonal control, anatomy, pharmacokinetics, and metabolism. A reproductive toxicant in one species may not be toxic in another (including humans) because of differences in reproductive or toxicologic mechanisms (see *Methoxychlor*). This complicates further the assumptions needed to extrapolate reproductive effects from experimental animals to humans. Another example is the difference in sensitivity of rats and mice to oocyte destruction by polycyclic hydrocarbons (Mattison, 1979; Mattison et al., 1979). This appears to be due to differences in pharmacokinetics rather than pharmacodynamics (Takizawa et al., 1985).

One critical issue in reproductive toxicology concerns possible gender differences in reproductive vulnerability. This is of concern because of gender differences in anatomy and biologic control mechanisms for reproduction. Because of the ease of access to gametes and gonads, xenobiotics may be tested for toxicity to male reproductive processes more readily than to female reproductive processes. Therefore, in the absence of data to the contrary, the working group feels that it is essential to assume that males and females are equally sensitive to reproductive toxicants.

Another critical issue in reproductive toxicity is windows during which sensitivity to toxicity occurs. This has been demonstrated in studies of developmental toxicants, toxicants acting on spermatogenesis, and toxicants acting on ovarian follicular development. Several studies have demonstrated that compounds like galactose or azathioprine are toxic prenatally, but have little effect postnatally (Mattison, 1981a; Mattison and Nightingale, 1982a) or disrupt reproduction by mechanisms in the adult different from those in the fetus (Swartz and Mattison, 1988). A small number of experimental and clinical studies have explored the effects of age on gonadal sensitivity to chemotherapeutic agents or other xenobiotics (Mattison, 1980; Kay and Mattison, 1985). Because reproduction is essential for the continuation of any species, it is important to develop an understanding of the mechanisms of action, sites of action, as well as species susceptibility to reproductive toxicants.

Direct-Acting Reproductive Toxicants

Structural Similarity One mechanism of action of direct-acting reproductive toxicants (Table 6-2) results from structural similarity to a biologically important molecule (e.g., hormone). These compounds may be thought of as biologic imposters, in that they mislead biologic processes. The

TABLE 6-2. Examples of Direct-Acting Reproductive Toxicants

Mechanism	Compound	Species	Gender	Selected References
Structural similarity	Steroid hormones	Mammalian	M,F	Harrington et al. (1978)
	Cimetidine	Primate	M	Winters et al. (1979) Van Thiel et al. (1979b) Wolf (1979)
	Diethylstilbestrol	Mammalian	M,F	McLachlan et al. (1975) Forsberg (1969, 1972) Vorherr et al. (1979) Boylan (1978) Bibbo et al. (1975, 1977) Kaufman et al. (1977) Haney (1979) Herbst et al. (1971, 1980) Schmidt et al. (1980) Barnes et al. (1980) Korach et al. (1979)

TABLE 6-2. Examples of Direct-Acting Reproductive Toxicants (Continued)

Mechanism	Compound	Species	Gender	Selected References
Structural similarity (cont'd)	Azathioprine, 6-mercaptopurine	Mammalian	M, F	Reimers & Sluss (1978) Reimers et al. (1980) Githens et al. (1965) Gross et al. (1977) Mattison et al. (1981)
	Halogenated polycyclic hydrocarbons	Mammalian	M, F	Kimbrough (1974) Kupfer (1975) Orberg & Kihlstrom (1973) Linder et al. (1974) Welch et al. (1969) Derr & Decker (1979) Nowicki & Norman (1972) Fahim et al. (1968, 1970) Poland et al. (1970) Kolmondin et al. (1969) Bitman & Cecil (1970)
	Galactose	Mammalian		Chen et al. (1982) Kaufman et al. (1981)

TABLE 6-2. Examples of Direct-Acting Reproductive Toxicants (Continued)

Mechanism	Compound	Species	Gender	Selected References
Chemical reactivity	Alkylating agents	Mammalian	M, F	Hemsworth & Jackson (1962, 1963) Jackson et al. (1959) Heller et al. (1964) Chapmann et al. (1979a, b) Sieber & Adamson (1975) Himmelstein-Braw et al. (1977, 1978) Hsu et al. (1979) Parra et al. (1978) Ettendorf et al. (1976) Oishi & Hiraga (1979) Fairley et al. (1972) Freeman-Narrod & Narrod (1977)
	Cadmium	Mammalian	M, F	Gunn & Gould (1970) Johnson (1977) Dixon et al. (1976) Parizek (1960, 1964) Parizek et al. (1968)

TABLE 6-2. Examples of Direct-Acting Reproductive Toxicants (Continued)

Mechanism	Compound	Species	Gender	Selected References
Chemical reactivity (cont'd)	Boron	Primate	M	Krasovski et al. (1976) Lee et al. (1978) Weir & Fisher (1972) Dixon et al. (1976)
	Lead	Mammalian, primate	M, F	Wide (1980) Jacquet et al. (1977)
	Mercury	Mammalian	M, F	Lamperti & Printz (1973, 1974) Lamperti & Niewenhuis (1976)

xenobiotics in this category are generally agonists or antagonists of endogenous hormones. Well-known examples of this type of direct-acting "reproductive toxicant" are the oral contraceptives, which act by suppression of preovulatory gonadotropin surges. Occupational exposure to oral contraceptives--for example, during formulation--represents an obvious example of a reproductive toxicant (Harrington et al., 1978). Occupational or environmental exposure to xenobiotics that are distributed to the hypothalamus or pituitary and act like estrogen, progesterone, or testosterone will similarly alter gonadotropin secretion and gonadal function (see FEMALE REPRODUCTIVE TOXICANTS).

Chemical Reactivity Compounds that are chemically reactive may be nonspecific in their site of action. Exposure to reactive chemical compounds generally occurs in an occupational or drug treatment setting. Examples of chemically reactive reproductive toxicants include alkylating agents, used in the chemical industry and in the treatment of some neoplastic diseases and other diseases. Because of chemical reactivity, most of these compounds are cytotoxic, carcinogenic, or mutagenic; and their reproductive toxicity has often been overlooked. The reproductive system, however, may be more sensitive to the toxic effects of these compounds than other organ systems or biologic end points. For example, the risk of sterility following many forms of cancer chemotherapy is considerably higher than the risk of second tumors (Kay and Mattison, 1985). In addition, in experimental animals, disruption of reproductive function occurs at doses much lower than those required to produce tumors. Other examples of chemically reactive direct-acting reproductive toxicants include metals such as cadmium, lead, and mercury, which are toxic to both the developing and mature reproductive system (Mattison, 1983).

Indirect-Acting Reproductive Toxicants

Metabolic Activation In addition to direct interference with reproduction, certain xenobiotics may act indirectly (Table 6-3). Examples include xenobiotics that are metabolized to chemically reactive products or products that mimic endogenous molecules. One of the mechanisms utilized to remove hydrophobic xenobiotics from the body is oxidation by microsomal monooxygenases, which increases the polarity of the molecule (Anders, 1985). Polar metabolites are then conjugated or excreted directly. Some of the metabolites formed in this process are chemically reactive. These reactive metabolites may interact with cellular macromolecules and produce reproductive toxicity similar to that observed following treatment with direct-acting chemically reactive compounds. The ovary and testis have been demonstrated to have microsomal monooxygenases, epoxide hydrases (Sims and Grover, 1974), and transferases responsible for metabolic processing of

TABLE 6-3. Examples of Indirect-Acting Reproductive Toxicants

Mechanism	Compound	Species	Gender	Selected References
Metabolic activation	Diethylstilbestrol	Mammalian	F	Blackburn et al. (1976) Rudiger et al. (1979) Metzler (1976) Metzler & McLachlan (1978)
	Ethanol	Mammalian	M, F	Van Thiel et al. (1979a) Anderson et al. (1980) Hinckers (1978) Ouellette et al. (1977) Jones & Smith (1975)
	Chlorcyclizine	Mammalian	M	Wong & Hruban (1972)
	Dibromochloropropane	Mammalian	M	Torkelson et al. (1961) Whorton et al. (1977) Marshall et al. (1978) Moody et al. (1980) Teramoto et al. (198O)

TABLE 6-3. Examples of Indirect-Acting Reproductive Toxicants (Continued)

Mechanism	Compound	Species	Gender	Selected References
Metabolic activation (cont'd)	Polycyclic aromatic hydrocarbons	Mammalian	M, F	Mattison (1980) Mattison & Thorgeirsson (1978, 1979) Wyrobeck & Bruce (1975) Nebert (1981) Felton et al. (1978) Basler & Rohrborn (1976)
	Cyclophosphamide	Mammalian	M, F	Koyama et al. (1977) Sirus et al. (1976) Sobrinho et al. (1971) Warne et al. (1973) Kumar et al. (1972) Rohrborn & Hansmann (1971) Uldall et al. (1972) Lentz et al. (1977)

TABLE 6-3. Examples of Indirect-Acting Reproductive Toxicants (Continued)

Mechanism	Compound	Species	Gender	Selected References
Hormonal disruption	Salicylazo-sulfapyridine	Mammalian	M	Levi et al. (1979) Traub et al. (1979) Toth (1979a, b)
	Halogenated hydrocarbons	Avian and mammalian	M, F	Kimbrough (1974) Kupfer (1975) Heinrichs et al. (1971) Gellert et al. (1973, 1974, 1978) Allen et al (1979) Iatropoulos et al. (1976) Lucier & McDaniel (1979) Thorpe (1967,1969) Welch et al. (1971) Gellert (1978) Gellert & Heinrichs (1975)
	Anticonvulsants	Primate	M, F	Aronson & Grahame-Smith (1981)

many xenobiotic compounds (Mattison and Thorgeirsson, 1978, 1979; Dixon and Lee, 1980; Heinrichs and Juchau, 1980). Metabolism to reactive products is responsible for the reproductive toxicity of cyclophosphamide, dibromochloro propane, and polycyclic aromatic hydrocarbons.

Hormonal Disruption Other indirect-acting reproductive toxicants may induce or inhibit hepatic or gonadal enzyme systems, enhancing or suppressing the secretion or clearance of steroids needed to control reproduction. Because reproduction requires hormonal feedback loops for successful control, xenobiotics that alter the rate of steroid synthesis or clearance may alter reproductive processes. This has been demonstrated in rodents treated with several of the halogenated hydrocarbon pesticides, including DDT, polychlorinated biphenyls, and polybrominated biphenyls, and suggested following human exposure.

Another class of reproductive toxicants may enhance rather than impair fertility. The effectiveness of oral contraceptives relies on feedback inhibition of gonadotropin secretion to prevent the ovulatory surge. Xenobiotics that stimulate clearance of the estrogenic and/or progestogenic component of oral contraceptives will decrease circulating levels and increase the probability of a gonadotropin surge and ovulation. This effect may be especially crucial in an occupational setting where other xenobiotics are teratogenic or fetotoxic.

Clinical Manifestations Suggesting Human Reproductive Toxicity

There are a series of human diseases of the hypothalamus, pituitary, ovary, and uterus that can be used to characterize observations suggesting female reproductive toxicity (Table 6-4). This table of clinical syndromes can be used to evaluate case reports or small clinical studies.

One example of an occupational exposure that produces easily understood and readily characterized reproductive toxicity is synthetic sex hormones (Table 6-5). Men and women working in the pharmaceutical industry exposed to dusts containing agonists or antagonists to estrogens, androgens, or progestogens, depending on the level of exposure, may have altered reproductive competence and decreased fertility. This direct effect through interaction with steroid hormone receptors is generally reversible with cessation of exposure and clearance of body burden.

Agricultural chemicals, including organochlorines and organo-phosphates, have been suggested as reproductive toxicants (Table 6-6). The organochlorines have been implicated in abnormal menses and impaired fertility. The effect of some of the organochlorines is thought to be direct, through interaction with estrogen receptors. Other organochlorines are

TABLE 6-4. Clinical Correlates of Ovarian Toxicity Produced by Xenobiotic Compounds

			Clinical Features		
Pathophysiologic Basis	Human Syndromes	Puberty	Amenorrhoea	Oocyte/Follicle Number	Follicle Profile
Toxicity in the developing ovary					
Oogenesis	Turner's	Delayed	Primary	Decreased	-
Oogenesis or follicle differentiation	Gonadal dysgenesis	Delayed	Primary	Decreased	-
	Premature ovarian	Delayed or normal	Primary or secondary	Decreased	-
Toxicity in the developed ovary					
Follicular growth	Hypogonadotropic hypogonadism	Delayed or normal	Primary or secondary	-	Abnormal
	17-β-Hydroxylase deficiency	Delayed	Primary	-	Abnormal
	"Resistant" ovaries	Delayed or normal	Primary or secondary	-	Abnormal

TABLE 6-4. Clinical Correlates of Xenobiotic Ovarian Toxicity (Continued)

			Clinical Features		
Pathophysiologic Basis	Human Syndromes	Puberty	Amenorrhoea	Oocyte/Follicle Number	Follicle Profile
	Alkylating agent therapy	Delayed or normal	Primary or secondary	Decreased	Abnormal
Ovulation	Polycystic ovary	Normal	--	--	Abnormal
	Hypothalamic-pituitary dysfunction	Normal or delayed	Primary or secondary		
Corpus luteum	Inadequate luteal phase	Normal	--	--	--
Oocyte or follicle	Premature ovarian failure (ionizing radiation, antitumor chemotherapy)	Normal or delayed	Primary or secondary	Decreased	--

TABLE 6-5. Clinical Manifestations, Site, and Mechanism of Reproductive Impairment from Occupational Exposure to Sex Hormones

Compound	Clinical Manifestation	Proposed Site	Proposed Mechanism
Androgens	Altered menses	Hypothalamus, pituitary, ovary	Direct androgen receptors
Oral contraceptives		Hypothalamus, pituitary, uterus	Direct estrogen and progesterone receptors
Mestranol, ethynylestradiol	Altered menses, intermenstrual bleeding	Hypothalamus, pituitary, ovary, uterus	Direct estrogen and progesterone receptors

TABLE 6-6. Clinical Manifestations, Site, and Mechanism of Reproductive Impairment from Occupational Exposure to Agricultural Chemicals

Compound	Clinical Manifestation	Proposed Site	Proposed Mechanism
Organochlorines (DDT, dichlorophenoxyacetic acid, hexachlorobenzene, lindane, toxaphene)	Abnormal menses, menorrhagia, amenorrhea, temporary infertility	Hypothalamus, pituitary, ovary, uterus	Direct (estrogen receptors) Indirect (prohormone hepatic induction)
Organophosphates	Abnormal menses, menorrhagia, amenorrhea, early menopause	Hypothalamus, pituitary, uterus, ovary	Indirect (unknown mechanism)

thought to be indirect-acting reproductive toxicants by virtue of being metabolized to estrogen agonists (Kupfer, 1975; Kupfer and Bulger, 1979) or induction of microsomal enzymes involved in the metabolism and elimination of steroids. It has been suggested that the organophosphates can disrupt normal reproductive function by producing abnormal menses, and even premature menopause. The site of action and mechanism of these effects, if true, remain to be defined.

Prenatal Reproductive Toxicants Compounds that produce prenatal ovarian toxicity in humans and experimental animals are summarized in Table 6-7. Our understanding of the site and mechanism of action of these compounds is limited, and they are presently identified by secondary effects resulting from absence of, or a considerable decrease in the number of, oocytes (e.g., primary or secondary amenorrhea).

TABLE 6-7. Prenatal Ovarian Toxicants

Compound	Endpoint	Species	Reference
Galactose	Fertility (oocyte number)	Human Rodent	Chen et al. (1982) Kaufman et al. (1981)
Benzo(*a*)-pyrene	Fertility (oocyte number)	Rodent	Mackenzie & Angevine (1981)
		Human (possibly)	Felton et al. (1978) Dobson et al. (1978) Mattison & Nightingale (1982b)
Ionizing radiation	Fertility (oocyte number)	Human Rodent	Mandl (1964) Ash (1980)
6-Mercaptopurine	Fertility	Rodent	Reimers & Sluss (1978) Reimers et al. (1980) Mattison et al. (1981)

Human syndromes associated with abnormal formation of the ovary and decreased numbers of oocytes are listed in Table 6-8. These syndromes demonstrate the clinical manifestations that would be observed in a population of women exposed prenatally to a xenobiotic substance that blocked germ cell migration, germ cell proliferation, urogenital ridge formation, oogonial differentiation, or folliculogenesis. Depending on the extent of oocyte destruction, or blockage of oocyte proliferation produced, the individuals will experience prepubertal, peripubertal, or postpubertal ovarian failure (Thomford et al., 1987). Morphologically, the ovary will be composed predominantly of stroma and have only a small number of follicles. Circulating levels of gonadotropins (FSH and LH) will be elevated, and ovarian hormones (estrogen and progesterone) will be decreased. In the absence of oocytes, these individuals will be infertile and, depending on the age of ovarian failure, may fail to reach all or some of the pubertal milestones.

TABLE 6-8. Human Syndromes Mimicking Prenatal Ovarian Toxicity

Human Syndrome	Clinical Features	Reference
Galactosemia	Amenorrhea, infertility (+/-), hypergonadotropic hypogonadism	Kaufman et al. (1981)
Premature Ovarian Failure	Amenorrhea, infertility (+/-), hypergonadotropic hypogonadism	Rebar (1982)

Prepubertal Reproductive Toxicants Ovulation and ovarian hormone production require interactions between the hypothalamus, pituitary, and ovary. Higher centers of the central nervous system may also play a role in modulation of the hypothalamic-pituitary-ovarian axis by integrating exogenous and endogenous stimuli. As ovarian function impacts directly on endometrial events that are readily apparent both to the patient and the physician (e.g., vaginal bleeding), ovarian and endometrial function are frequently discussed simultaneously. Accordingly, prepubertal reproductive toxicity is best elucidated at the present time by using a clinical approach that examines the impact of chemicals on menstrual cyclicity (see Table 6-5).

The hypothalamus integrates a variety of stimuli, including circulating levels of ovarian hormones, and responds with the release of gonadotropin releasing hormone (GnRH) (Knobil, 1980). At the present time, it is thought that GnRH permissively controls the release of both the gonadotropins, FSH and LH. Prepubertally, the hypothalamus secretes little GnRH and as a result there are infrequent pulses of FSH and LH (Styne and Grumbach, 1978). The pituitary, however, is capable of responding to GnRH during this period with secretion of adult levels of gonadotropins (Wildt et al., 1980). During the prepubertal period the frequency of GnRH pulsation increases and the frequency of gonadotropin pulsation also increases.

The ovary, like the pituitary, appears fully competent shortly after birth and responds to the increased stimulation by gonadotropins with increased estrogen secretion. Finally, in the sexually mature organism, the GnRH pulse frequency and amplitude is such that it permits integrated functioning of the pituitary and ovary (Carmel et al., 1976).

Shortly after birth, in rodents and primates, including humans, an elevation of FSH occurs (Fuller et al., 1982). Suppression of this FSH increase in rodents by lead or mercury has been associated with subsequent dysfunction of the reproductive system (Mattison, 1983). Other xenobiotics, including diethylstilbestrol (DES), certain halogenated aromatic hydrocarbons, and other estrogen agonists, can produce similar hypothalamic dysfunction in rodents. As the role of elevated FSH may differ considerably in rodents and primates, it is not known if prepubertal exposure to these compounds would alter subsequent reproductive function in humans (Mattison, 1981a). Prenatal treatment of women with DES appears to exert its major adverse effects on the structure of the developing vagina, cervix, and uterus with minimal effects on the functioning of the hypothalamus and pituitary (Mattison, 1981a).

Other factors may also modify the age-dependent sensitivity of the reproductive system. The prepubertal rodent ovary is more sensitive to cyclophosphamide than the adult gonad (Mattison et al., 1983). Age-dependent alterations in reproductive tract sensitivity to other toxicants, including metals, may also occur. Possible mechanisms for altered resistance of the prepubertal gonad may reside in a decreased rate of gonadal cell proliferation (Mattison, 1981a) or alterations in the distribution of the toxicant to gonadal cells (Setchell and Sharp, 1981; Janson, 1975). For some toxicants, the prepubertal gonad may be more sensitive than the sexually mature gonad (Kay and Mattison, 1985). As an example, the level of glutathione is lower in the immature rodent gonad than in the mature gonad (Mattison et al., 1983). Xenobiotics detoxified by glutathione may be more toxic to the maturing than to the mature gonad. The relationship between changing ovarian glutathione and prepubertal cadmium toxicity is not well understood (Kar et al., 1959).

Adult Reproductive Toxicants The clinical assessment of reproductive toxicity can be an extremely difficult task. An instructive example of this difficulty occurs in patients exposed prenatally to DES. A host of studies have examined the male and female DES progeny, with conflicting results (Mattison, 1981a, 1981b). Careful analysis of the data suggests that impaired reproductive function can occur in exposed female offspring and might occur in exposed male offspring. A good data base is provided by the offspring of women who were participants in a double-blind study in which both drug and placebo groups were matched. Even with this study design, subtle defects that impair fertility may be difficult to characterize. Similar well-matched groups will be difficult to obtain in settings of occupational or environmental exposure.

In addition, the time of evaluation of the selected end point may influence the outcome. Xenobiotics that alter hypothalamic patterning in rodents may not produce adverse reproductive effects until the attainment of sexual maturity (Mattison, 1981b). Reproductive toxicants might not alter functioning in the nonstressed organism, but might produce profound alterations in the reproductive performance of the stressed animal. For example, the dynamic reproductive function of the nonpregnant animal might be normal, but the animal might be infertile because it cannot adapt to the physiologic or metabolic demands of pregnancy. Such a mechanism might explain the increased rate of spontaneous abortion among female metal workers over 30 years of age compared with those younger than 30 (Hemminki et al., 1985).

The experiments of Sunderman et al. (1985) demonstrating decreased fetal weight and hematocrit in offspring of female rats treated with nickel subsulfate before breeding also suggest that toxicity from exposures before pregnancy can alter subsequent reproductive performance. As increasing numbers of women are delaying their first pregnancy for schooling or work, the effect of chemical exposures or toxicity prior to pregnancy on subsequent metabolic adaptations to pregnancy becomes an increasing concern. Because of these observations, it is necessary to devise a variety of testing paradigms that evaluate reproductive performance in young and old, healthy and diseased, stressed and nonstressed animals.

Just as end points or sites for reproductive toxicity may be difficult to evaluate, the site or sites of toxic interaction may also be elusive. Because interactions along the hypothalamic-pituitary-ovarian-uterine axis are dynamic, impaired function may result from direct toxicant interaction with one of these organs (Mattison, 1981b). For example, DES, a potent estrogen agonist, can impair reproduction in the sexually mature female by inhibiting FSH and LH release at the level of the hypothalamus and pituitary. Several halogenated hydrocarbons are also potent estrogen agonists and can alter hypothalamic function (Kupfer, 1975; Kimbrough, 1974). Impaired reproduction may also result indirectly as a result of metabolic alterations that disrupt hormonal interactions. This mechanism of action has been suggested for polycyclic aromatic and halogenated hydrocarbons (Mattison, 1981b; Welch et al., 1969, 1971) and also appears to account for the decreased effectiveness of oral contraceptives and increased clearance of- estrogens in patients using anticonvulsants (Aronson and Grahame-Smith, 1981). Despite all of these caveats, many xenobiotic compounds have been implicated in reproductive dysfunction with sites of action along the hypothalamic-pituitary-ovarian-uterine axis. The pesticides evaluated in this chapter demonstrate the vulnerability of both the male and female reproductive systems to insult. The effects of pesticides on reproductive processes can also be evaluated by comparison with human syndromes of reproductive dysfunction. These and other experiments of nature represent defined or partially characterized disorders of reproduction that provide a data base of clinical and endocrinologic parameters essential for evaluation of reproductive toxicants (Mattison and Ross, 1982).

MALE REPRODUCTIVE TOXICANTS

Dibromochloropropane

Introduction Dibromochloropropane (DBCP) is the pesticide most toxic to the human male reproductive system that has been studied to date. DBCP is a brominated organochloride and was used as a nematocide from the mid-1950s until its ban in the late 1970s (Whorton and Foliart, 1983). This pesticide was used to protect citrus, grapes, peaches, pineapples, soybeans, and tomatoes. In 1975, 25 million pounds were being produced in the United States (Whorton and Foliart, 1983). Most of the DBCP produced in the United States was used along the southern Atlantic and Pacific coasts and Hawaii.

Unfortunately, the history of our knowledge of the reproductive toxicity of DBCP is not a good example of hazard identification and risk management. Data available in the literature since 1961 should have prevented the adverse effects later observed in humans. A study reported in 1961 (Torkelson et al., 1961) indicated that DBCP caused testicular atrophy in rats, guinea pigs, and rabbits. Similar data were also reported in the rat in Russian papers in the early 1970s (Faidysh et al., 1970; Rakhmatullaev, 1971). This information was essentially ignored until 1977 when Whorton et al. reported azoospermia and oligospermia in DBCP production workers. Several other reports of human reproductive effects were published before more comprehensive animal studies were conducted to study dose-response relationships and mechanisms of action of this potent toxicant. DBCP induces adverse genetic, testicular, endocrine, and possibly secondary sex gland effects in the male reproductive system.

Testicular Effects The testicular and endocrine effects are related and will be discussed together. Extensive reports exist demonstrating that azoospermia and oligospermia are related to the length of time working with DBCP (Whorton et al., 1977; Marshall et al., 1978; Potashnik et al., 1979; Sandifer et al., 1979; Lipschultz et al., 1980; Milby and Whorton, 1980). Many of the oligospermic and most of the azoospermic men also had FSH levels above the normal range. In followups of exposed workers, those men whose FSH levels remained high did not have spermatogenetic recovery. The high levels of FSH are probably part of the normal endocrine feedback response to disrupted spermatogenesis and not a primary effect of DBCP on the neuroendocrine system. The disruption of spermatogenesis is further demonstrated by testicular biopsies of exposed men (Whorton et al., 1979b) who showed, in severe cases, a lack of spermatogonia, with a pathology similar only to Sertoli cell syndrome. In less severe cases, the biopsies indicated a decrease in the cellularity of the seminiferous tubules.

Data from animal studies reveal the stage and severity of the testicular insults. Torkelson et al. (1961) reported that rats exposed to 12 ppm of DBCP by inhalation for 50 exposures (7 hours/day, 5 days/week) had degenerative changes in the seminiferous tubules. These changes included an increase in Sertoli cells, a reduction in the number of sperm cells, and an increase in abnormal morphologies of sperm cells. Kluwe (1981b) observed epididymal and testicular toxicity daily after a single subcutaneous (sc) injection of 100 mg DBCP per kg of body weight (bw) in F344 male rats. On the first day he noted epithelial cells in some seminiferous tubular lumens and in epididymal tubules. Also in five of six animals, caput epididymal epithelial cells were vacuolated. By day 2, there was mild focal seminiferous tubular degeneration with desquamation of all germinal stages. Progressive degeneration of seminiferous tubules was observed from days 3 to 5, with the number of sperm in the epididymal tubules being sparse or absent.

Posttesticular Effects The animal studies of Kluwe et al. (1983b) reported reduced fertility in rats 2 to 7 days after a single sc injection of 100 mg/kg of DBCP. Due to the rapid onset of the observed response, this could not be a spermatogenic insult, but instead a posttesticular event. Bartooov et al. (1987) also reported that DBCP inhibits sperm motility *in vitro* in freshly ejaculated ram sperm.

Germ Cell Line Genetic Changes DBCP has been reported to increase chromosomal exchanges in rat spermatogonial tissue (Kapp 1979) and to produce dominant lethal effects in rats, but not in mice (Teramoto et al., 1980; Rao et al., 1983). The genetic effects in humans are based on the double Y-body fluorescence test (YFF test) and the pregnancy outcomes fathered by exposed workers. Kapp et al. (1979) reported a significant increase in the number of sperm with the YFF classification in semen from exposed compared with unexposed men. He hypothesized that the YFF classification was an assay for Y chromosomal nondisjunction. However, recent sperm karyotyping studies indicate that Y chromosomal nondisjunction prevalence is in reality much lower than that measured by the YFF test (Martin 1985). This assay may still be useful in indicating an alteration in the Y-bearing spermatozoa because studies indicate higher prevalence of female births after fathers were exposed to DBCP (Potashnik et al., 1984; Goldsmith and Potashnik, 1984). Whorton (1982) reported increases in the incidence of spontaneous abortions in females whose husbands were exposed to DBCP. In rats, Amann and Berndtson (1986) reported an increase in embryonic loss in pregnancies attributed to DBCP-exposed males at levels at which fertility was normal (oral dose, 15 mg/kg daily for 77 days). Goldsmith and Potashnik (1984) and Potashnik and Abeliovich (1985) reported no increase in the spontaneous abortion rates in pregnancies attributed to exposed men. Potashnik and Abeliovich (1985) studied the children fathered by men who were exposed to DBCP in the workplace. The chromosomal constitutions of children conceived during or after DBCP exposure were normal. The children were also reported to be in excellent health and lacked malformations.

Mechanisms Kluwe (1981b) determined that the rat was an appropriate model for studying the mechanism of DBCP as a gonadal toxicant. His conclusion was based on testicular lesions observed in rats that received a single sc injection of 100 mg/kg of DBCP, which are similar to the lesions seen in the testes of the exposed worker. Warren et al. (1984) has reported that DBCP, and not a contaminant of the production grade DBCP, is the reproductive toxicant. The mechanism of action of DBCP is at the level of mitochondrial respiration. The studies of Kluwe et al. (1983a) also indicated that DBCP (as the parent compound) plays a role in the subsequent testicular toxicity. Miller (1986) reported that pyruvate-supported mitochondrial respiration was inhibited by DBCP *in vitro*. Furthermore, Miller reported that DBCP stimulated lactate production in cultured rat Sertoli cells and inhibited aerobic metabolism of lactate by cultured spermatocytes, which resulted in spermatocyte but not Sertoli cell cytotoxicity. He went on to hypothesize that DBCP, because of its effects on mitochondria, is selectively cytotoxic to immature germ cells due to their dependence on aerobic metabolism. Therefore, DBCP-induced mitochondrial dysfunction disrupts spermatogenesis, at least in the rat. He also indicated that DBCP, and not its metabolites, was responsible for this mechanism of action. Greenwell et al. (1987) studied DBCP effects on mitochondrial metabolism in the mature sperm cell of the rat. They were able to determine that DBCP inhibits carbohydrate metabolism at the NADH dehydrogenase step in the mitochondrial electron transport chain of rat sperm. This would explain the rapid onset of infertility in rats after a single dose of DBCP.

Dose Response To evaluate risk, it is important to determine the dose response to the toxicant. Torkelson et al. (1961) reported only a single concentration (inhalation of 12 ppm, 66 exposures, 7 hours/day, 5 days/week) for the rabbit and guinea pig, but clearly demonstrated a dose-response effect of DBCP on testes weight in the rat at concentrations of 0, 5, 10, and 20 ppm (50 exposures, 7 hours/day, 5 days/week). Kluwe (1981a) found that the severity of injury in the rat to the kidney, epididymis, testis, and liver was proportional to dose. Warren et al. (1984) demonstrated dose-related decreases in both testis weight and body weight using pure DBCP and production grade DBCP (96.8% pure, with allyl chloride as the major contaminant). Amann and Berndtson (1986) reported dose-response effects on body weight, weight of the epididymis, daily sperm production, and number of sperm in the epididymis in rats exposed to DBCP orally at doses ranging from 0.0 to 15.0 mg/kg each day for 77 days. Even more important is the dose response seen in human males. The longer a man worked with DBCP, the lower was his sperm count (Whorton et al., 1977; Whorton et al., 1979b). The likelihood of recovery from azoospermia also appears to be related to length of time working with DBCP (Potashnik, 1983). Goldsmith and Potashnik (1984) also provide data that suggest that the sex ratio of female to male offspring is related to length of human exposure to DBCP.

Summary The data on DBCP demonstrate that the fertility problems observed in humans could have been predicted from existing animal data. This scenario supports not only the need to test pesticides and other potential reproductive toxicants in well-designed animal studies, but also to take heed of the results.

Chlordecone

Introduction Chlordecone, better known by its registered trade name Kepone™, was first synthesized in 1951 and patented by Allied Chemical and Dye Corporation in 1952. Reel and Lamb (1985) detailed the use and eventual ban of chlordecone. The following is a brief summary of their report. Chlordecone was manufactured from 1958 to 1975 and sold as an agricultural insecticide and fungicide. Production was stopped in 1975 after reports of dose-related neurotoxicity in production workers. Chlordecone from the plant in Virginia had also contaminated the James River and its tributaries. In 1976, the U.S. EPA cancelled the registration of 12 pesticide products containing chlordecone.

Animal Studies Early studies of chlordecone did not suggest that the chemical was a severe male reproductive toxicant. Huber (1965) fed male mice 40 ppm of chlordecone impregnated into the chow for 6 months. At the end of the treatment, the mice were mated to untreated females. There was a decrease in litter size but no abnormalities were noted in the histologic analysis of spermatogenesis or the interstitial cells of the treated males.

Since the human toxicity data was released (Taylor et al., 1978), several other animal studies have been conducted to determine possible reproductive effects in the male. Larson et al. (1979) reported testicular atrophy in rats fed 50 ppm of chlordecone for 3 months. A dermal study in rabbits was conducted by applying a bran bait containing 2% chlordecone to shaved skin (Epstein 1978). The quantities of chlordecone applied were equivalent to 5 and 10 mg/kg body weight. Animals were treated 8 hours a day, 5 days a week, for 3 weeks and testicular atrophy resulted in two of six rabbits at the 5 mg/kg dose and in one of six rabbits at the 10 mg/kg dose.

Hammond et al. (1978) studied the reproductive effects of both chlordecone and Mirex by feeding rats a diet containing 30 ppm of either chlordecone or Mirex (an insecticide that photodegraded to chlordecone) (Carlson et al., 1976). Chlordecone increased testosterone levels in the fourth week of feeding and decreased seminal vesicle weight in week 8. Serum LH fluctuated irregularly; and in the seventh week of feeding chlordecone, the litters fathered by exposed animals (mated to untreated females) were smaller than the controls. The only effect of Mirex was an increase in seminal vesicle weight in weeks 1 and 2. Cannon and Kimbrough (1979) studied the effects of chlordecone on Sherman strain rats. The animals were fed 0 or 25 ppm of chlordecone in the diet for 3 months. Male reproduction was not affected by chlordecone exposure at these doses. Linder et al. (1983) exposed male rats to diets containing 0, 5, 15, or 30 ppm chlordecone for 90 days. They reported that there were no effects on reproductive performance nor were there histologic changes in the reproductive organs of treated males. They did note, however, that epididymal sperm motility and viability were decreased in the animals dosed with 15 and 30 ppm. Simon et al. (1986) studied the distribution of chlordecone in the reproductive tracts of male rats given a single oral dose of 40 mg/kg of ^{14}C-labeled chlordecone. The distribution of the label, in descending order, was seminal vesicular fluid > prostate > vas deferens > seminal vesicle (organ itself) > unwashed sperm > washed sperm.

Mechanism of Action Because chlordecone is a potential inducer of the mixed function oxidase system, Hammond et al. (1978) hypothesized that chlordecone may affect fertility by stimulating hepatic degradation of steroids. Chlordecone is readily hydrated, and the hydrate appears to be the active molecule in biologic systems (Guzelian 1982). Chlordecone (probably as the hydrate) has a binding affinity for estrogenic sites. The binding affinity of chlordecone is about 0.01 (Hammond et al., 1979) and the estrogenic potency is about 0.001 (Eroschenko and Palmiter, 1980) compared with 17-estradiol. Even these low amounts of chlordecone can have pronounced effects, due to its long half-life and bioaccumulation in the body (Reel and Lamb, 1985).

Cochran and Wiedow (1984) concluded that the mechanism of the reproductive toxicity of chlordecone was not a mimicry of estrogen. This was based on their study in which they compared the effects of estradiol at 1.4, 1.9, 2.4, or 3.6 mg/day and chlordecone at 180, 260, 340, 500, or 600 mg/day (via a capsule implant) for 72 days in male rats. While several adverse reproductive effects were noted with the estradiol treatment, none were seen in the chlordecone-treated animals. It should be noted that in this study estradiol caused a decrease in seminal vesicle weight and in sperm motility. Both of these effects have been reported in some, but not all, of the other animal studies of the reproductive toxicity of chlordecone. A study by Hong et al. (1985) reported that a single intraperitoneal (i.p.) injection of 75 mg/kg of chlordecone into male rats reduced the pituitary content of enkephalin in a manner identical to that of estrogen. This demonstrates the effect of chlordecone on the rat hypothalamic-pituitary axis.

Human Studies Taylor et al. (1978) reported that production workers (n = 21) exposed to chlordecone were oligospermic and had reduced sperm motility together with other symptoms. Cohn et al. (1978) reported that 19 of 20 men who had blood chlordecone levels higher than 1,000 ng/ml had motile sperm counts less than 25 million/ml. They reported that cholestyramine reduced the level of chlordecone in the blood and that in 12 of 13 men, the number of motile sperm/ml increased as the blood chlordecone levels decreased. This indicates that the effects of chlordecone on sperm motility are reversible.

Summary It appears that chlordecone primarily affects sperm motility and viability. These variables, which are related, are rarely evaluated in toxicity studies, and if they are, the assessments are very subjective. This could be a possible explanation for the inconsistent reports of the effects of chlordecone on male reproduction. It also appears that the mechanism of action is at least in part due to estrogenic-like properties of the hydrated form of chlordecone.

Ethylene Dibromide

Animal Studies Ethylene dibromide (EDB, 1,2-dibromoethane) has been found to produce stomach tumors in rats and mice, hepatic nodules and cancer in female rats and hemangiosarcoma in male rats, and respiratory tract tumors in mice (NCI, 1979). EDB also adversely affects the male reproductive system.

Because of the widespread use of EDB as a feed and grain fumigant, much attention has been focused on the effects of this compound on large animal reproduction. Amir and Volcani (1965) reported that EDB administration to bulls caused changes in sperm morphology; the timing of the effect suggested that developing testicular spermatids were one target. Many subsequent studies confirmed this finding (Amir and Volcani, 1967; Amir, 1973, 1975; Amir et al., 1977, 1979, 1983). This was also confirmed ultrastructurally in bulls (Courtens et al., 1980) and rams (Hrudka and ElJack, 1979). The bull study used peritesticular injection, small numbers of animals (one or two per time point), a single dose, and collected testes by serial castration. The authors concluded that specific steps of elongating spermatids were sensitive to the effects of EDB. There were numerous changes in nuclear morphogenesis, structures surrounding the nucleus (the perinuclear ring and perinuclear substance), and acrosomal development. Ultrastructurally in the only testis examined at this time point, dark deposits were seen on day 3 in many testicular cells, including the affected cell types. These deposits were interpreted as bromine residues, but this was not evaluated by elemental analysis.

Another ultrastructural study (Hrudka and ElJack, 1979) used rams treated subcutaneously for 12 days with 13.5 mg EDB/kg bw per day. The animals (number unspecified) were orchiectomized on days 22, 27, 32, and 38 after the start of dosing. Ultrastructural changes were seen relating primarily to the acrosome in cap-phase spermatids. The authors concluded that the subsequent changes in spermatid nuclear metamorphosis and shaping were related to the lack of proper acrosomal development, which led to abnormal nuclear condensation and Sertoli manchette formation. Another study pointing to the particular susceptibility of the spermatids is that of Amir and Tadmor (1981), who reported that peritesticular injection of radiolabelled EDB into bulls resulted in an accumulation of the label in the testicular spermatids. Curiously, they reported no radiolabel accumulation in testes from rams treated similarly, a finding they interpret as consistent with the insensitivity of the ram to the "spermatotoxic" effects of EDB. It is difficult to reconcile this conclusion with the obvious effect demonstrated by Hrudka and ElJack (1979).

The ultrastructural studies cited above provide compelling morphologic evidence of damage, but the use of these data for human risk estimation is impaired by the choice of test species, the small numbers of animals, and the unusual route of administration. Unfortunately, there are few studies in laboratory species focused on the reproductive effects of EDB, although there are many studies on the genotoxicity and the metabolic processes required to produce the proximal tumorigen.

Short et al. (1979) report an inhalation study where CD rats were exposed to 0, 19, 39, or 89 ppm of EDB 7 hours per day, 5 days per week for 10 weeks. Treated males and females were mated to untreated animals and reproductive performance monitored by sacrifice of the females in midgestation. All animals were killed for histologic evaluation of reproductive tissues. The only effects in the males were seen at the highest doses where none of the animals were fertile. However, the mean body weight for this group was 35% less than the control mean, and the investigators did not control for this severe body weight loss. Thus, the complete infertility, testicular atrophy, epididymal atrophy, and decrease in size and weight of prostate and seminal vesicle in this dose group cannot be interpreted. There was no reproductive histopathology identified in females.

Hurtt and Zenick (1985) conducted a study to evaluate the effects of EDB exposure in rats (0, 22, or 44 mg EDB/kg/day). Animals were gavaged for 12 or 30 days and killed 24 hours after the last dose. The high-dose group showed evidence of impaired spermatogenesis by day 12 (decreased epididymal weight and sperm count and decreased testicular spermatid count) and increased liver and kidney weight. By day 30 in the high-dose group, these changes were greater and seminal vesicle weight was also decreased. These end points were unaffected in the low-dose group, with the exception of testicular spermatid count, which was decreased on day 30. In Bouins-fixed paraffin sections, there were no histopathologic lesions in the low-dose group, and only "moderate" lesions in some of the high-dose rats. Thus, in this study the quantitative measures were more sensitive. This study is important because it documents changes in rodent spermatogenesis in the absence of body weight changes; it also confirms that spermatids are one affected cell type in the testis. This study was not designed to provide extensive dose-response information or to identify the principal affected cell type in the testis, when this change would be first seen, or the extent of any involvement of the accessory sex organs.

Sega and Sotomayor (1980) also found that spermatids contained most of the label after injection into rats of ^{3}H-EDB. The radiolabel was not found attached to DNA; the authors speculated that the label was binding to protamines. While this study corroborates the spermatid target cell data reviewed above, the lack of histologic evaluation means that the timing of germ cell maturation in the testis could have been affected, which would change the conclusion about the target cell type. It is likely that a small dose of EDB (5.2 mg/kg) would not have changed this timing, but it was not evaluated.

The decrease in seminal vesicle weights reported by Hurtt and Zenick (1985) is provocative. Many reproductive toxicants can affect spermatogenesis without modifying androgen status or secondary sex organ weight. The accessory sex organs most commonly evaluated in toxicologic studies (prostate and seminal vesicle) depend heavily on circulating testosterone to maintain their secretory capacity (review by Coffey, 1988). Because these secretions make up such a large percentage of the weight of the organs, this end point (prostate and/or seminal vesicle weight) has been commonly used to provide a general indication of circulating androgen status. Thus, when secondary sex organ weights are decreased by treatment, the next step usually is to treat some additional animals and measure serum testosterone. The decreased organ weights reported by Hurtt and Zenick (1985) imply an effect of EDB either on the hypothalamic-pituitary-Leydig cell pathway controlling testosterone secretion or on the accessory sex organs themselves. It is important that this finding be further evaluated. Because no chemicals have yet been shown to have a direct effect on accessory sex organ function in the absence of hormonal effects, the demonstration of such an action for EDB would be unique and exciting. Alternatively, a lesion of the central systems controlling LHRH--and thus LH and testosterone secretion--would be important to document because androgen status is more closely regulated than is spermatogenesis by the neural control systems in the hypothalamus and pituitary. Thus, altered androgen production could mean that the brain and pituitary are targets for EDB or that the target site could be limited to the interstitial cells in the testis.

At least some involvement of the interstitial cells is suggested by the autoradiographic data of Kowalski et al. (1985), who reported that testicular interstitial cells have the ability to activate EDB to "metabolites that become bound to the tissue." Whether or not these metabolites interfere with the ability of the Leydig cells to produce and secrete testosterone is another unaddressed question.

The testicular interstitium contains most of the cytochrome P450 in the testis (Mukhtar et al., 1978b). Both microsomal (smooth endoplasmic reticuluum) and mitochondrial P450 are involved in the synthesis of testosterone (review by Payne et al., 1985). EDB can be metabolized by two routes *in vivo* (Guengerich et al., 1980; review by Sipes et al., 1986). Oxidation (via P450) and dehydro-bromination form bromoacetaldehyde, a compound that binds to cellular macromolecules, primarily microsomal proteins *in vitro*, and is not mutagenic (Shih and Hill, 1981; Bannerjee and van Duuren, 1979; Hill et al., 1978; Rosenkranz, 1977). However, conjugation with glutathione (GSH) produces a stable N7-guanine DNA adduct (Inskeep et al., 1986). Glutathione depletion decreases unscheduled DNA synthesis (UDS) observed in hepatocytes after exposure to EDB *in vivo* or *in vitro* (Working et al., 1986). Conversely, inhibition of P450- oxidation of EDB caused an increase in the UDS response in both hepatocytes and in spermatocytes. This suggests that the P450-mediated reaction serves as a clearance pathway, while the GSH-dependent pathway is linked with genetic damage (Working et al., 1986; White et al., 1983). It is noteworthy in this context that EDB is unable to produce dominant lethal effects in male rodents (Short et al., 1979; Teramoto et al., 1980).

The findings that progeny from EDB-treated sires have altered central neurotransmitter and behavioral patterns (Hsu et al., 1985; Fanini et al., 1984) suggest that EDB exposure produces changes in the sperm that have functional consequences for the offspring. The conclusion of Fanini et al. (1984) that EDB affects premeiotic germ cells may seem to be at odds with the previous demonstrations of structural defects in late postmeiotic cells. This might be due to differences between species, or it might represent the difference between observable structural defects and occult, functional changes.

While it appears that most of the P450 in the testis is interstitial, and much of the glutathione is intratubular (and probably in germ cells) (Mukhtar et al., 1978b), the fate of EDB in the testis is unclear. Both the interstitium and the germ cells appear to have the metabolic capabilities to transform EDB, and to transform it by potentially different mechanisms. Inskeep and Guengerich (1984) hypothesize, based on both their data and those of others, that the amount of DNA binding is the result of the balance between the detoxification and activation processes. How these are balanced *in vivo* remains an important unanswered question.

There are more questions than answers about where and how EDB affects reproduction. We do know that:

- Spermatids appear affected in large animal species;
- Spermatogenesis is adversely affected in rats;
- There may be secondary sex organ involvement in rodents; and
- Two different pathways of metabolism exist, making products with potentially dissimilar fates *in vivo*.

We do not know:

- The range of functionally or structurally affected cell types in the rodent testis;
- Which testicular cell type is most sensitive to EDB (affected at the lowest concentration);
- The dose-response relationships for EDB effects on these cells;
- The nature of involvement of the accessory sex organs in rodents;
- The extent of central involvement in the reproductive lesion;
- Which metabolites are responsible for the spermatogenic damage and their site of production (i.e., local activation or sequestration from the blood);
- The relative species sensitivity to EDB; or
- Which end point is most sensitive in test animals, a reproductive effect or a tumorigenic response.

Human Studies Several studies in the literature have attempted to evaluate the effects of EDB on the human male reproductive system. Of these, three are difficult to interpret due to choices of design or analysis or to questions of sensitivity.

Ter Haar reported in 1980 on an effort to monitor the fertility and other health effects of workers exposed to EDB. The author concluded that EDB had no adverse effect on the reproductive or general health of the exposed men, but these conclusions were disputed due to the small number of subjects and the time from exposure to evaluation that might have been insufficient for a full tumorigenic response to occur.

One study (Takahashi et al., 1981) performed a limited semen analysis on men exposed to EDB during agricultural applications. Although this paper documents a decrease in the mean and median sperm count per ml ejaculate and an increase in abnormal forms, the lack of exposure data, the insufficiency of statistics, the lack of controls for confounding variables, the small number of participants, and the heterogeneous control population all make this a difficult study to interpret.

Wong et al. (1979) reported on a retrospective evaluation of the fertility of the wives of exposed workers. Although this study did attempt to include controls for some confounders, the fact that the authors did not determine actual exposure levels of EDB or perform a semen analysis, coupled with the recognition that the end point (expected fertility rate) is relatively insensitive, raises doubts about the strength of the conclusions. Nonetheless, the finding that the wives of men at one of the four manufacturing facilities had a lower than expected fertility rate increased the urgency that a controlled semen evaluation of EDB-exposed men be performed.

A recent report by Ratcliffe et al. (1987) may fill the gap somewhat. This paper also evaluated agricultural EDB applicators, but was unique in including exposure monitoring, detailed questionnaire and sample collection instructions, and a thorough statistical analysis that acknowledged and controlled for 11 possible confounding variables. The semen analysis consisted of videomicroscopic end points (motility, velocity), semen volume, pH, sperm viability, morphometry, morphology and count, as well as the presence of YFF bodies. This analysis found that in men exposed to a time-weighted average exposure concentration of 88 ppb of EDB, there were decreases in sperm count per ejaculate, an increase in specific sperm abnormalities, and a decrease in the viability and motility indices. This study appears to address the appropriate confounders and used appropriate statistical approaches in data analysis by recognizing, for example, that multiple comparisons increase the chances of a false positive result. Thus, the level of significance for the sperm abnormalities was set at $p < 0.001$ to decrease the possibilities of false positives. Using this conservative approach, the exposed men had significant increases in the percentage of sperm with tapered heads, no heads, and abnormal tails.

The Ratcliffe et al. (1987) study stopped short of evaluating whether these altered semen parameters produced changes in the fertility of the affected men. This highlights one of the many difficulties of human studies, which is working with a sample size that is too small to identify an effect in a very "noisy" end point like reproduction. It would also be desirable to repeat this study with another population. This would be difficult to do, given the decreasing use of EDB and the more stringent management of exposure.

EDB provides an interesting example of a situation where the animal reproductive data base has some large holes, and several studies demonstrate or suggest effects in humans. More frequently, the opposite is true. A careful longitudinal analysis in laboratory animals of the effects of EDB on sperm production, morphology, and motility, as well as accessory sex organ involvement, might be of immense benefit as a "positive control" for extrapolating the response from experimental animals to humans.

Carbaryl

Carbaryl (N-methyl-1-naphthyl carbamate; Sevin^R) is a carbamate insecticide used for its ability to inhibit acetylcholinesterase by the carbamylation of the active-site serine residue (Murphy, 1980). Because of its widespread use, human exposure potential is high, both at application and by the consumption of treated food (WHO, 1967).

Animal Data Carbaryl appears to be a weak mutagen in drosophila and does not induce genomic deletions or affect male fertility (Brzheskii, 1972; Brzheskii and Vaskov, 1971). Carbaryl does not decrease the incorporation of radiolabelled thymidide into murine testicular DNA, an effect that is thought to correlate with mutagenicity (Seiler, 1977). Although direct injection of an aqueous preparation of carbaryl into grasshopper abdomen (in effect, a peritesticular injection) produced "a tendency for chromosomal clumping in the spermatocytes" (Venkat-Reddy et al., 1974), the real significance of this effect is not known. The route and species are unusual, the choice of preparation is questionable (carbaryl is not readily soluble in water), the dose was unknown, and the biologic significance of these small changes for the gametogenic capabilities of the grasshopper testis was not addressed.

Although Lillie (1973) reported that supplementing the diet of Leghorn chicken hens with up to 500 ppm of carbaryl decreased the weight gain of the offspring, this diet had no effect on rooster sperm abnormalities or embryonic abnormalities in young sired from treated males. Also, DeRosa et al. (1976) found that after addition of carbaryl to the diet of Coturnix quail, carbaryl was detected in the egg yolk, and egg production was decreased. The treated females showed more aggressive behavior, while the treated males showed less. Offspring behavior was not monitored.

Unlike ethylene dibromide, carbaryl does not appear to be metabolized to an active intermediate; the parent compound is thought to be the active agent *in vivo*. The complex metabolic route to detoxification has been reviewed by Fukuto (1972) and Ryan (1971). Although an epoxide is a postulated requisite intermediate based on some of the structures found as metabolites, it does not appear that this binds to, or reacts with, cellular macromolecules in the same way that the half-mustard of EDB does. Many of the initial reports of reproductive toxicity of carbaryl have come from the Soviet Union and are discussed in Weil et al. (1972). These studies report adverse effects of carbaryl on rodent spermatogenesis at doses ranging from 0.4 to 5 mg/kg administered *per os* or intraperitoneally. The relative inaccessibility of these reports (most are printed in Russian) makes them difficult to evaluate. In addition, subsequent studies published in the United States (below) call into question the replicability of the initial findings.

Shtenberg and Rybakova (1968) treated "albino rats" with 7, 14, or 70 mg carbaryl per kg of body weight per day for up to 12 months. No attempt was made to provide controls for the significant weight loss in the 14 and 70 mg/kg dose groups. As an additional index of exposure, there was a dose-related decrease in the activity of blood acetyl-cholinesterase activity. The authors showed that sperm from treated rats could not maintain their motility as long as sperm from controls could. Although the authors report testicular histopathology, the published pictures are difficult to interpret because (1) no picture of controls is shown and (2) the "depression of spermato genesis" and "interstitial oedema" appear within normal levels of artifact produced by the histologic methods used. Using an unspecified method of assaying pituitary function, they determined that there was increased gonadotropin secretion from the pituitaries of treated animals. Although not clearly stated in the paper, it appears that animals in all doses showed this increase. The authors conclude that these central changes are primary and that peripheral end-organ effects are secondary. There are not enough data or methodologic details provided to allow the reader to verify this.

Jordan et al. (1975) administered 20 mg carbaryl/kg i.p. to male mice once and sacrificed the animals 10 or 20 days later. *Inter alia*, testes, brain, and pituitary were examined for "karyometric" changes in 10 μ-thick sections after fixation in Bouin's fixative and paraffin embedding. Using these methods, no effects on the testes were observed. The authors did report an increase in the volume of the cells in the mediobasal hypothalamus and a decrease in the size of hepatocytes. Although the hypothalamic change may suggest the involvement of central systems that control the gonad, this was not addressed; and the significance of the findings was not discussed. Indeed, by themselves, these data can only suggest a hormonal component in the carbaryl lesion.

If these "effects" were of sufficient intensity and duration, one would expect that the reproductive capacity of the treated animals would eventually be decreased. However, while there were a number of toxicity studies performed with carbaryl in the late 1960s, most were teratology studies (Weil et al., 1972); very few addressed potential male reproductive injury by carbaryl. Most of the available information comes from fertility studies.

In a three-generation fertility and reproduction study, Collins et al. (1971) reported on rats fed a diet containing up to 10,000 ppm of carbaryl. At 2,000 ppm, there was a decrease in weight only in weanlings, whereas at 5,000 ppm, additional effects were seen in the average number of animals weaned and in the survival index. At 10,000 ppm, in addition to the changes seen for the lower dose groups, there was complete infertility after the first litter in the second generation. The highest concentration adversely affected survival, fertility, viability, and lactational measures. Gerbils were slightly more affected than rats at these doses. This study was not designed to evaluate which sex was preferentially affected by carbaryl, so the reader cannot determine if the males were affected by the exposure.

For comparison across studies, we might approximate the daily consumption in the Collins study as follows: a 250-g rat will eat 15 to 20 g of food per day. The high dose (10,000 ppm) has 10 mg of carbaryl per gram of feed. Thus, 15 g of feed containing 150 mg of carbaryl was consumed by an animal weighing 0.25 kg, so the dose was 150 x 4 = 600 mg/kg. If this is true, then the group fed the lowest dose consumed approximately 120 mg/kg, and the middle dose group consumed approximately 300 mg/kg. Because these figures will vary from laboratory to laboratory and with the weight and age of the animal, they are only approximations, but might be used for comparative purposes.

The other major study of subchronic carbaryl administration to rats (Weil et al., 1973) found no effect on fertility when the animals were fed diets containing sufficient carbaryl to provide them with up to 200 mg/kg/day. This dose is in the range of the low dose used by Collins et al. (1971), although no data were provided to ascertain that this level of intake was reached. At 200 mg/kg/day, an increase in the time between mating and delivery of the first litter occurred in the second generation only. A concurrent gavage test group found effects at the highest level of gavaged dose (100 mg/kg/day) on the median number of pups born alive in the second and third generation and also a decrease in the percentage of females that littered. This latter effect was present in the second generation, but not in the third, and this end point was not changed in the diet-route study even at 200 mg/kg/day. These data led the authors to conclude that the effects of carbaryl were slight and required a gavage route of administration. The untested implication is that the different pharmacokinetic profile (higher peak serum level) produced after a bolus administration by gavage was critical in producing the reproductive toxicity.

Additionally, in the Weil et al. (1973) study, the males from the third litter in the second generation were serially mated to untreated females, who were killed and examined midway through gestation. There was no evidence of treatment-induced dominant-lethal effects. Microscopic examination of the reproductive organs from an unstated number of males from the third generation showed no evidence of histopathology in the reproductive system (Weil et al., 1973). Whether the contemporary methods would have detected a moderate decrease in spermatogenesis is unknown. In fact, because neither of the two multigeneration studies report organ weight, testicular spermatid head count, or epididymal sperm count, morphology, or motility data, significant (≤ 50% to 60%) diminutions in spermatogenesis could have occurred unnoticed. Nonetheless, given these data, the authors concluded that carbaryl had no effect on rodent reproduction.

A similar conclusion was reached by Dikshith et al. (1976b) who gavaged four groups of rats (n = 7 per group) with 0 or 200 mg/kg/day carbaryl, 3 days/week for 90 days. They reported changes in some testicular enzyme activities but did not explain why the changes were seen and did not address the significance of the effects. They reported no evidence of histopathology, although the techniques used produced sections with considerable artifacts that would have obscured subtle changes in spermatogenesis. Males from this dosing regimen were mated each with two females, and the authors reported no effect on fertility. Although the numbers of males were too small to provide adequate statistical power for the fertility study, and although those histologic methods can hide numerous effects, the authors' conclusion that carbaryl is not a potent reproductive toxicant is probably valid. This is supported by two pieces of data: (1) the presence of sperm in epididymal lumena suggests that testicular output was not severely compromised; and (2) the absence of immature germ cells in the epididymis indicates that the testis was not damaged so badly that it was releasing cells prematurely. However, the quality of the sperm cannot be confidently assessed by this study design; although a large dominant-lethal effect could be seen, a mild effect would not be expected to show up using such a small number of animals. Additionally, because no quantification of sperm number or output was made, small-to-moderate diminutions in spermatogenesis would have been undetected. Thus, although we may believe that carbaryl is not a potent male reproductive toxicant, we cannot conclude from this study that carbaryl did not affect spermatogenesis in the treated rats.

Studies by Thomas and co-workers (Thomas et al., 1974) were designed primarily to compare the effects of carbaryl against those pesticides with demonstrable binding to the steroid receptor in accessory sex organs. Their studies indicate that carbaryl does not affect the response of these accessory sex glands to androgens.

Many of the above studies required substantial doses for extended periods of time to observe effects on fertility. Nonetheless, it is noteworthy that little has been done to identify where in the reproductive system those effects occurred. Those studies that have evaluated potential sites or mechanisms have focused on the central hormonal control pathways (Shtenberg and Rybakova, 1968) or on peripheral androgen-sensitive target tissues (Thomas et al., 1974). Little or no evaluation is available on the process of spermatogenesis, testicular structure in impaired animals, or sperm morphology.

From the available studies, we can derive that:

- Carbaryl is a very weak mutagen in the fruit fly;
- Carbaryl does not induce dominant-lethal mutations in male rodents; and
- Consumption of 120 to 200 mg/kg bw per day in the diet is the approximate threshold for minimally affecting rodent fertility.

The studies above do not tell us:

- Whether the males in the fertility studies were affected, or if the fertility impairment was an effect primarily on the female;
- The affected cell type(s) in the testis;
- The degree of involvement of the central control systems (carbaryl's neurotoxicity makes this a possible etiologic factor);
- The degree of involvement of the accessory sex organs; or
- The dose-response relationships between exposure to carbaryl and effects on sperm production, motility, or morphology. Indeed, the lack of sperm assessments are particularly noteworthy in light of the human data.

Human Studies There are two studies available on the results of exposure of human males to carbaryl. Both used the same group of men involved in the production and packaging of the pesticide.

The first study was reported by Whorton et al. (1979a), who evaluated sperm count from one semen sample per subject. In the absence of rigorous exposure characterization data, the authors ranked the subjects into three exposure categories. Assignation to one of these categories depended on the type of job and how frequently one worked at that task. Length of time (months, years) at that task was also factored into the analysis. Fertility also was evaluated where possible. Because no concurrent control group could be recruited, the authors used historical controls from an "external control group." The constraints on controls and sample handling (length of time from collection to analysis) forced the authors, finally, into evaluating only the distribution of sperm counts. The exposed and historical control sample groups did not differ significantly in the distribution of their sperm counts.

Subsequently, the same group of men was evaluated by Wyrobek et al. (1981). Concurrent controls were available for this study, and the contribution of such confounding variables as smoking, "positive medical histories," age, and known exposure to other toxicants was evaluated. Exposure was characterized in a manner similar to that used by Whorton et al. (1979a), after acknowledging the values for carbaryl in the air collected by the employer's industrial hygiene monitoring program as varying between 0.36 and 14.21 mg/m^3 (mean = 4.9 mg/m^3). The statistical methods were different from those used by Ratcliffe et al. (1987) in the evaluation of EDB-exposed men, but more rigorous than commonly found. The level of significance (0.05) in the Wyrobek study was also different from that used by Ratcliffe et al. (1987) ($p < 0.001$ for sperm morphology), thus increasing the chances of finding an effect.

While Wyrobek et al. (1981) found no exposure-related change in sperm number per ejaculate, the exposed men had significantly more morphologically abnormal sperm. The biggest difference, taking confounders into account, was found between exposed and control men in the 18- to 40-year-old category, where the controls had a mean of 42% abnormal sperm (± 2.7%, SEM), and the exposed men had 56% abnormal sperm (± 4.3%, SEM). This effect was not "dose" related and was inversely related to the number of years of working with carbaryl (i.e., men working fewer than 6 years had more abnormalities than did those working longer).

The data of Wyrobek et al. (1981) suggest that carbaryl exposure, no matter how poorly documented, had an effect on human semen. In view of this finding, another study of carbaryl-exposed men is critical for confirming the link between exposure and effect. An absolute requirement for any future study is adequate documentation of exposure. It would also be useful to include in the semen evaluation additional end points, such as motility and viability.

The question of whether the human male is more sensitive to carbaryl than the rodent cannot be addressed without being severely hindered by data gaps. One major frustration is that different end points have been assessed in different species: fertility in rodents, sperm morphology in humans. Rodent fertility is very robust (Amann, 1982), while sperm morphology is thought to be considerably more sensitive (Wyrobek et al., 1981). We are also hampered by the lack of relevant and satisfying human exposure data on which to base this extrapolation.

An additional question is whether the changes in sperm morphology (approximately 10% increase in abnormal forms) reported by Wyrobek et al. (1984) translate into impaired fertility in the affected men. Wyrobek et al. concluded their report by drawing together some indirect evidence that supports the link between abnormal sperm shape and heritable defects in the offspring. Much of this is from the animal literature, where a female component can be more effectively ruled out (e.g., Soares et al., 1979). Although humans are necessarily less manipulable, human data do exist that are consistent with this hypothesis (reviewed by Wyrobek et al., 1981, 1983, 1984). However, the finding that morphologically abnormal human sperm do not function as well as normal sperm in the zona-free hamster egg penetration test (Marsh et al., 1987) suggests that there will not be more heritable genetic damage, but perhaps fewer fertilized ova, and thus, impaired fertility. In their study of carbaryl workers, Wyrobek et al. (1981) found no fertility effect, but the sensitivity of the study was quite low because the number of subjects was small. Because carbaryl is still produced and widely used, it should be feasible to identify a cohort of exposed men of sufficient size to fill the data needs identified above, attempt to replicate the findings above, and at least address the question of altered function.

In summary, the rodent studies indicated that high-dose/long-term exposures were required to see fertility effects with carbaryl. On the other hand, rodent gametogenesis and fertility require large percent changes to be manifest functionally (i.e., there needs to be a large decrease in sperm number to show up as a change in fertility), and some of the potentially more sensitive end points (motility, morphology) were not assessed in the rodent studies. Thus, we must conclude that subtle effects on spermatogenesis could indeed have occurred. Such effects were found by Wyrobek et al. (1981) in humans. The available human studies lack good exposure characterization and statistical power. Thus, although the data suggest that carbaryl does not produce a testicular effect similar to DBCP, one cannot be sure that production personnel are unaffected by the chemical. Only additional well-designed studies can answer that question.

FEMALE REPRODUCTIVE TOXICANTS

Female Toxicity Due to Estrogenicity

Introduction Certain similarities exist between the chemical structures of the organochlorine insecticides and those of both the natural estrogen 17ß-estradiol and the synthetic estrogen DES. The estrogenic properties of the insecticide DDT have been studied most extensively. Presumably, structural similarities enable certain compounds to interact with cellular receptors or enzymes with sufficiently high affinity to exert biologic effects. These effects need not be related to the ability of a compound to interact with nucleoprotein.

Estrogenic pesticides theoretically can affect multiple estrogen-dependent processes in the female reproductive system at different stages of development. Determination of gender, which occurs *in utero*, could be altered by compounds able to traverse the placenta. Sexual development, particularly of the brain, could be altered by neonatal exposure to estrogenic pesticides. The onset of reproductive function could be advanced by exposure to estrogenic pesticides, so that puberty might occur early (this has been demonstrated in rodents). Finally, exposure to estrogenic pesticides during adulthood could lead to aberrant hypothalamic-pituitary-ovarian function, with effects of hyperestrogenism at multiple loci. Estrogenic effects could include sensitization of the pituitary gland to GnRH, inhibition of pituitary gonadotropin secretion, alteration of ovarian follicular development, and stimulation of the organs of the reproductive tract (e.g., uterus, cervix, and vagina).

DDT Porter and Wiemeyer (1969) examined the effect of a mixture of DDT (4.7 ppm) and dieldrin (0.84 ppm) in the diet on eggshell thickness in sparrow hawks. The combination of insecticides had the greatest effect in the first generation offspring of treated females. These yearlings had 15% thinner eggshells than controls. Black ducks fed DDE at 10 ppm for two breeding seasons produced eggs with approximately 20% thinner shells than untreated controls (Longcore and Stendell, 1977). Cessation of DDE feeding led to progressively thicker eggshells. Robson et al. (1977) did not observe any effect of DDT (100 ppm in the diet for six consecutive 28-day periods) or DDE (100 or 300 ppm in the diet for eight periods) on eggshell thickness in Japanese quail. McCluskey et al. (1984) compared the fates of 656 eggs of mountain bluebirds, western bluebirds, and house wrens from an area sprayed with DDT (0.84 kg/hectare) with the fates of 711 eggs from a control area. They found no difference between the two groups. The inconsistent effects of DDT and DDE on eggshell thickness might reflect different sensitivities among species of birds. Although the positive effects that were observed might seem minor, the thinner eggshells are associated strongly with disappearance of eggs and increased destruction of eggs (Porter and Wiemeyer, 1969). Numerous researchers have hypothesized that eggshell thinning in DDT-treated birds is a function of the similarity between DDT and estrogen, since estrogen inhibits formation of eggshells.

DDT has pronounced uterotropic effects in birds and mammals, but again, there are different sensitivities to the insecticide among species. In three generations of beagle dogs treated with up to 10 mg/kg/day orally, the only reproductive effect observed in treated females was a 2- to 3-month advancement of puberty (Ottoboni et al., 1977). Bitman et al. (1968) unequivocally demonstrated that *o,p'*-DDT (3 intraperitoneal injections of 50 mg) increased weight (100%) and glycogen content (150% to 175%) of the oviducts of Cornish chickens and Japanese quail. Glycogen content of uteri of immature rats treated with 4 mg of *o,p'*-DDT increased (> 3.5-fold in 18 h) in a manner very similar to that of animals treated with 0.4 μg estradiol (Bitman et al., 1968).

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In immature rats, treatment with 500 μg *o,p'*-DDT daily for 27 days advanced the time of vaginal opening and increased the weight of the ovaries and uteri (Gellert et al., 1972). Treatment of mature ovariectomized rats with 10 mg/day of *o,p'*-DDT or its metabolite *p,p'*-DDA for 7 days increased uterine weight and produced a thickened, vacuolated uterine epithelium; it also induced cornified vaginal smears and reduced serum LH concentrations (Gellert et al., 1972).

The response of the uterus to estradiol involves a series of events that begin with binding of hormone to cellular receptors and result in increases in cellular progesterone receptors, tissue mass, DNA synthesis, and cell division. Such estrogenic effects on uterine cells have been demonstrated for DDT. Mason and Schulte (1980) reported that treatment of ovariectomized adult rats with 10 mg *o,p'*-DDT for 3 days doubled the number of progesterone receptors in uterine cytosol, the same effect as 1 μg estradiol. Ireland et al. (1980) showed that *o,p'*-DDT (250 mg/kg) increased uterine DNA content and DNA synthesis in ovariectomized immature rats to the same extent as 40 μg/kg of estradiol. The effects of the DDT and estradiol were not additive, suggesting that both acted by the same mechanism, presumably mediated via the estrogen receptor. Nelson (1974) demonstrated that *o,p'*-DDT displaced [^{3}H]estradiol from binding sites in rat uterine cytosol with an EC_{50} of 0.6 ppm (2 μM). The increase in uterine weight observed after treatment of immature rats with 10 to 1,000 mg/kg *o,p'*-DDT appears to be mediated via estrogen receptors since levels of nuclear estrogen receptor correlate closely with increases in uterine weight measured 3 and 24 hours after insecticide administration (Robison and Stancel, 1982). Nuclear estrogen receptor levels also correlated closely with the amount of induced protein formed (Robison et al., 1984). Finally, Haney et al. (1984) reported that *o,p'*-DDT mimicked the ability of estradiol to suppress progesterone production by porcine granulosa cells in culture. They found that *p,p'*-DDT had the same effect as *o,p'*-DDT, whereas few other investigators have found *p,p'*-DDT to mimic the effects of estradiol.

DDT appears to affect neuroendocrine differentiation and function. Treatment of rats on days 2 and 3 of life with as little as 0.1 mg of *o,p'*-DDT induced persistent vaginal estrus and anovulation in adulthood (Heinrichs et al., 1971; Gellert et al., 1974). Etgen (1982) demonstrated that treatment of ovariectomized rats with three injections of 200 mg/kg of *o,p'*-DDT mimicked the ability of three injections of 1 μg of estradiol to induce female sexual behavior. Treatment with the same dose for 15 days inhibited compensatory ovarian hypertrophy in unilaterally ovariectomized rats (Etgen, 1982).

Research in humans suggests that maternal body burdens of DDT/DDE may relate to preterm delivery and spontaneous abortion (Saxena et al., 1981; Procianoy and Schvartsman, 1981). Cord blood of preterm infants and placental tissue from spontaneously aborted conceptuses contain significantly higher levels of DDT and DDE than do comparable materials from term births, although levels in maternal serum are not consistently higher (O'Leary et al., 1970). One explanation for the higher levels of organochlorine pesticides in abortuses and preterm infants is that the estrogenicity of these compounds might precipitate labor.

The Working Group believes that no studies of the effects of exposure to DDT or DDE on menstrual function or fertility have been performed in human females. In theory, such lipophilic compounds might well influence steroid hormone levels.

Methoxychlor Methoxychlor is a chlorinated hydrocarbon insecticide with much lower acute toxicity than DDT. In rats, it is metabolized *in vivo* via *o*-demethylation by hepatic microsomes to a compound with estrogenic activity (Bulger et al., 1978b; Kupfer and Bulger, 1979; Bulger et al., 1985).

Methoxychlor has direct estrogenic effects on the rat uterus. Like estradiol, methoxychlor induces the enzyme ornithine decarboxylase (Bulger et al., 1978a). It also inhibits the decidual cell response of the hormonally conditioned female rat uterus at doses of 200 mg/kg of body weight per day administered during days 1-8 of pseudopregnancy (Cummings and Gray, 1987). The decidual cell response is accepted as a model for the events associated with blastocyst implantation, so it is conceivable that methoxychlor could decrease fecundity by preventing implantation. The degree of inhibition by methoxychlor was similar to that seen with a dose of estrone of more than 2.5 μg/rat/day.

Central estrogenic effects of methoxychlor have not been demonstrated. Treatment of neonatal female hamsters with a single injection of 1 mg of the insecticide did not alter later postpubertal estrous cyclicity, and it did not cause postpubertal male mounting behavior in response to testosterone (Gray et al., 1985); estradiol had both effects. Gellert and Wilson (1979) were unable to affect reproductive function in female offspring of rats fed methoxychlor during pregnancy. Failure to produce effects on differentiation of the hypothalamic-pituitary-ovarian axis might reflect inability of fetal or neonatal tissues to convert methoxychlor to estrogenically active metabolites.

Chlordecone It was observed as early as the 1960s that chlordecone (Kepone) and its completely chlorinated analog Mirex impair the reproductive performance of birds and mammals. DeWitt et al. (1962) reported that reproduction in bobwhite, quail, and pheasant was blocked if the birds were fed diets containing 5 to 10 ppm of chlordecone; lower pesticide concentrations were partially inhibitory. Similarly, 70 to 150 ppm of chlordecone and 600 ppm of Mirex in the diet reduced egg production in laying chickens (Naber and Ware, 1965). In mice, both chlordecone and Mirex reduced the number of offspring produced by females fed the pesticide one month before mating and during the test period (Huber, 1965; Ware and Good, 1967). The effect of chlordecone was dose-related; the number of young produced was reduced 23.9%, 79.3%, and 87.0% at 10 ppm, 30 ppm, and 37.5 ppm, respectively.

At a dietary concentration of 40 ppm, chlordecone caused a state of constant estrus in mice, with development of large follicles and absence of corpora lutea in the ovaries (Huber, 1965). McFarland and Lacy (1969) observed similar effects of chlordecone on the ovaries of Japanese quail. In animals treated with 200 ppm of chlordecone for 30 days, ovarian weight was greater than in controls because of increased numbers of developing follicles as well as increased numbers of large follicles.

Chlordecone appears also to have a direct estrogenic effect on the reproductive tract. McFarland and Lacy (1969) found that chlordecone (300 ppm) fed to ovariectomized quail increased the size of the oviduct, which exhibited hypertrophy, hyperplasia, and a secretory epithelium. Eroschenko and Becker (1984) reported that chlordecone (80 to 160 ppm) in the diet of immature quail caused similar changes, but in addition induced atypical ultrastructural characteristics such as protruding or swollen cellular apices, increased mitochondrial volume, and disorganized Golgi. The insecticide also stimulates the growth of the rodent uterus. Estradiol or increasing doses of chlordecone up to 125 μg administered daily to 1-day-old female mice caused keratinization of vaginal epithelium and hypertrophy, hyperplasia, and glandular formation in uterine epithelium (Eroschenko and Mousa, 1979). The reproductive tracts of neonatal mice nursed by dams that were being injected with up to 1,000 μg chlordecone daily showed similar signs of estrogenization (Eroschenko and Osman, 1986). The effect of chlordecone on uterine tissue probably is mediated via estrogen receptors since Hammond et al. (1979) showed that when the insecticide is injected into immature rats at doses as low as 6 mg/kg bw per day for 3 days, it increases uterine weight and stimulates synthesis of progesterone receptor; *in vitro*, it competes with estradiol for binding to uterine receptors with greater potency than *o,p'*-DDT and induces accumulation of estrogen receptor in the nucleus. One cellular manifestation of chlordecone's effect is selective enhancement of synthesis of uterine proteins (Heinz et al., 1987).

There is little information regarding the effect of chlordecone on central nervous system components of the reproductive system. Gray (1982) administered 2-day-old hamsters 0.5 or 1 mg of chlordecone, ovariectomized the hamsters postpubertally, administered testosterone, and observed subsequent sexual behavior. In contrast to controls, female hamsters treated neonatally with estradiol or chlordecone abnormally displayed male mounting behavior in response to a receptive female. These data suggested that chlordecone had an estrogen-like effect on the sexual differentiation of the hamster brain. Uphouse et al. (1986) found that in contrast to estradiol, doses of chlordecone as high as 75 mg/kg did not increase progesterone receptors in the brains of ovariectomized female rats, although the chemical did induce constant vaginal estrus.

Lindane The nonaromatic chlorinated cyclic hydrocarbon lindane has seemingly conflicting effects on the female reproductive system. Welch et al. (1971) reported that treatment of immature rats with 15 mg/kg bw per day for 7 days resulted in a 37% increase in estrone metabolism by liver microsomal enzymes. At the same time, lindane treatment reduced the increase in uterine weight in response to estrone 50%, presumably because of the decreased exposure of the uterus to estrone. At the above dose, lindane itself did not have a uterotropic effect.

Administration of lindane (20 mg/kg bw per day) to ovariectomized adult rats for 30 days caused 23%, 28%, and 61% increases in glycogen content (mg/100 g tissue) of vagina, uterus, and cervix, respectively (Raizada et al., 1980). In agreement with the results of Welch et al. (1971), however, lindane alone did not increase the weights of those organs or affect the response to estradiol dipropionate.

As with DDT, estrogenic effects of lindane might be expected to be mediated via estrogen receptors. However, Faria et al. (1984) were unable to demonstrate inhibition of binding of [^{3}H]estradiol to receptors at lindane concentrations as high as 10^{-4}M. These results suggest that lindane can affect female reproductive function by altering multiple processes. It appears that interaction of lindane with different non-genomic sites in lindane-sensitive tissues might account for multiple effects.

Risk Estimation The acknowledged difficulty in extrapolating the results of animal toxicity studies to humans lies in the significant differences in absorption, distribution, action, metabolism and excretion of pesticides among species. These differences, as well as the pronounced differences in reproductive success among species necessitate hazard characterization in at least three unrelated species with reproductive processes that parallel those of the human (e.g., mouse, rabbit, and pig). Such interspecies comparisons would decrease the likelihood of incorrectly concluding that a pesticide presents little or no reproductive hazard.

Future Directions There appears to be a lack of attention devoted to cellular and molecular mechanisms by which pesticides alter bodily functions. Current research seems to rely heavily on gross physiologic indices of reproductive hazard, and few pesticides exist for which the mechanism of toxicity is known at the cellular level. The estrogenic insecticides are one exception, in part because of the observation (Burlington and Lindeman, 1950) that the chemical structure of DDT resembles that of DES and estradiol, and in part because of the availability of established *in vitro* and cell-free end points of estrogen action.

Development of indices of reproductive hazard must be pursued vigorously. As with DDT, a pesticide can act at multiple loci. Pesticides should be screened using *in vitro* techniques that permit more discrete localization of their effects than *in vivo* techniques. Some of the processes that might be utilized as end points for identification of reproductive hazard in the female in addition to those discussed above are:

- Response of perifused hemipituitaries to GnRH;
- Response of cultured pituitary cells to GnRH;
- Expression of FSH and LH mRNA levels in the pituitary;
- Number of oocytes in the ovary;
- Expression of inhibin mRNA levels in the ovary;
- FSH receptor levels in ovarian granulosa cells;
- FSH activation of adenylyl cyclase in ovarian granulosa cells;
- FSH induction of aromatase activity in ovarian granulosa cells;
- FSH induction of cytochrome P450 side chain cleavage activity, i.e., increased progesterone secretory activity;
- FSH induction of LH receptors and LH-sensitive adenylyl cyclase; and
- Ovarian proteoglycan synthesis.

Ultimately, one must know the exact molecular mechanism by which a pesticide produces a deleterious effect. With DDT, at least one mechanism is the interaction of the insecticide with estrogen receptors.

Reproductive Effects Associated with Organophosphates and Carbamates

Organophosphates The early observation that the acute toxicity of parathion resembled excessive cholinergic stimulation and that the symptoms could be alleviated by atropine suggested an inhibition of acetylcholinesterase activity in the nerve tissues as the mechanism of toxic action (DuBois et al., 1948). This was later confirmed by the finding that the tissue of rats poisoned by parathion had high free levels of acetylcholine in the brain and reduced levels of cholinesterase activity (DuBois et al, 1949). Reviews of the central effects are available (Cohen and Oosterbaan, 1963; Heath, 1961; O' Brien, 1976).

The organophosphates have been associated in a number of species with a combination of a reduction in brain acetylcholinesterase activity (achase) and altered reproductive behavior. Some of the observed effects, however, indicated a gonadal target effect. This may be due to secondary influences on the central control of reproduction, although a direct action on the gonad cannot be excluded.

Reduced brain achase has been associated with decreased egg production and serum LH and serum progesterone. The reductions occurred in response to a dose of 25 ppm and 100 ppm in a dose dependent manner. In addition, there was an alteration in the response of the animals to cold stress which was associated with a 2 to 5 fold increase in the plasma corticosterone levels (Rattner et al., 1982a). In mice exposed to 50 and 100 mg/kg acephate, there was a reduction in the brain achase by 45 and 56% and in basal LH levels of 29 and 25% (Rattner and Michael, 1985). This suggests that acute exposure may have a role in the modification of LH activity. The dermal treatment of mice with 100 mg/kg cruformate on a chronic basis before and during two pregnancies showed that this agent reduced the conception number in both the first and second pregnancy. There was also a reduction in the reproduction period in the first pregnancy. Of interest, the brain achase levels dropped 96% 24 hours after the initiation of treatment (Khan, 1973). These findings have extended to the egg production of quail in response to parathion. Bobwhite quail fed parathion in concentrations of 30 to 400 ppm parathion were found to have a cessation of egg production, inhibition of follicle development and reduced serum LH levels at the 100-ppm dose (Rattner et al., 1982b).

A positive dominant lethal test has been reported in mice treated with methylmethanesulfonate orally at a dose of 100 mg/kg body weight. This was observed despite a negative dominant lethal test among similar mice treated with dichlorvos (Dean and Blair, 1976).

The direct application of agents to the egg has been used as a test of hatchibility. The application of metalodemeton to incubated eggs of the pheasant has shown a reduction in hatchibility (Kulczycki, 1980). In addition, the oral treatment of the chicks with this agent and formothion and demefion reduced the reproductive performance in terms of egg laying capacity (Kulczycki, 1980).

In the teleost, the chronic administration of fenitrothion at a concentration equal to the LC_{50} resulted in the reduction in ovarian weights after 120 days and a loss of mature follicles at a point of 150 days of administration (Saxena and Garg, 1978). Cythion has been shown to inhibit gonadal development when exposed to 2 mg/liter for six months. These changes were associated with a reduction in pituitary gonadotropin. The immature oocytes of the exposed fish demonstrated cytoplasmic proteinaceous inclusion bodies (Ram and Sathyanesan, 1987). These inclusions were seen to be present prior to the degeneration of these oocytes. This suggests that as well as a central site of action cythion may have an effect on the gonad directly. Further work in the teleost has shown that malathion, chlorfenvinphos, tetrachlorvinphos, and mevinphos were associated with a panorama of reproductive findings (Haider and Upadhyaya, 1985). The chronic exposure to sublethal concentrations resulted in the loss of stage II and stage III oocytes, a reduction in the gonadosomatic index, a lack of lipid yolks, and a reduction in PAS staining in the follicular layer. In addition, there was a reduction in both 3β-hydroxysteroid dehydrogenase and glucose-6-phosphate dehydrogenase activity.

It has been suggested that a possible mechanism of toxicity involves the interference of trichlorphon and parathion with steroid hormone binding to receptors in the liver, adrenal, uteri, and testes of the rat. The binding of testosterone and estradiol to uterine cytosol receptors has been shown to be reduced below control values (Trajkovic et al., 1981).

Carbamates

The carbamates are similar to the organophosphates as the mode of action is a reduction in acetylcholinesterase (achase) activities within the brain (Casida, 1963; O'Brien, 1967). In addition to the cholinergic effects which are produced by the poisoning, there is an anesthetic effect on the exposed subject.

Zineb has been shown in the rat to decrease the serum gonadotropins in response to oral administration for 16 days despite the fact that organophosphate agents (ethyl parathion and dimethoate) did not demonstrate a similar effect (Bentue-Ferrer et al., 1981). The exposure of rats to zineb and thiram has shown an alteration (prolongation) of the estrous cycle in association with a reduction in ovarian and uterine weights (Ghizelea and Czeranschi, 1973).

In the teleost, carbamate has been associated with a reduction in the number of oocytes, deformity of existing oocytes, increase in the rates of atresia, increase in the connective tissue of the *tunica albuginea*, and reduction of the gonadosomatic index (Kulshrestha and Arora, 1984). The effects were dose and duration dependent.

In a large variety of mammals exposed in the environment to 0.1 to 5.0 kg/ha, there was a significant reduction in fertility (Krylova et al., 1975). Among the species studied, there was a reduction in spermatogenesis, pathological pregnancy, increased embryonic resorption, increased proportion of infertile males and females, and an increase in males with underdeveloped testicles. In addition, a reduction in the number of embryos per pregnancy occurred (Krylova et al., 1975). As a functional test of the oocyte, carbamates have been associated with possible direct effects at this level. The administration of a carbamate to the mouse and rabbit oocyte inhibits the oocyte meiotic maturation, and prevents germinal vesicle breakdown and the formation of cytoplasmic microtubules near the nucleus. In addition, in the mouse oocyte exposed to isopropyl-*N*-phenylcarbamate, there is a formation of nuclear condensates which have been considered to be the aggregation of macromolecules (Crozet and Szollosi, 1979).

Summary

From this brief review of the literature regarding the organophosphates and the carbamates, several issues appear particularly relevant:

1. There are abnormalities of the hypothalamic-pituitary-ovarian axis in many species. The predominant characteristic is that of suppression of the axis.

2. These alterations are associated with a reduction of achase in the brain.

3. Important direct effects at the level of the gonad cannot be excluded in either class of pesticides.

4. An excellent rationale appears to be present for reviewing the possible effects of these agents among males and females.

Reproductive Toxicity of Kepone and Hexachlorobenzene

Two pesticides, chlordecone (Kepone) and hexachlorobenzene (HCB), have been evaluated for their effect on reproductive function in animal studies. The end points of reproductive toxicity analyzed in this summary are (1) fertility (measured experimentally as the number of litters or young produced relative to the number of females exposed to mating) and fecundity (measured experimentally as the size of the litter, and (2) cyclicity (in the case of lower laboratory animals, refers to the estrous cycle). Perturbation of cyclicity by toxic agents is usually monitored experimentally by the production of persistent vaginal estrus (PVE).

Kepone is a polycyclic chlorinated insecticide, and HCB is used as a pre-emergent fungicide. Although HCB is not produced as a pesticide, considerable amounts of this persistent compound are formed as a byproduct of other chemical formulations. Both compounds are ubiquitous in our ecosystem and have been found to disrupt the reproductive physiology of certain mammalian and avian species.

Animal Studies Kepone has been shown to produce adverse effects on both fertility and fecundity in mice. Doses of 5 and 10 ppm (Good et al., 1965) and 10-40 ppm (Huber, 1965) of Kepone in the diet 1-2 months prior to and 3-5 months following mating resulted in dose-related reductions in both the number and size of litters in Balb/c mice. Cannon and Kimbrough (1979) produced 100% infertile CDBS rats after feeding 25 ppm of Kepone in the diet for 3 months prior to mating. Partial restoration of reproductive ability (45% fertility) was observed 9 weeks after administration of the test diet.

Studies by Eroschenko and Palmiter (1980) demonstrated a reduction in fertility in Japanese quail fed 200 ppm of Kepone in the diet for 4 or 8 months. Reduction in the number of eggs laid (32% at 4 months and 70% at 8 months relative to controls) decreased number of intact eggs (46% at 4 months and 86% at 8 months compared with controls), and an increase in the egg-laying interval (2x for both time periods compared with controls) was observed in this study.

Both rats and mice are sensitive to the perturbation of cyclicity following various exposure regimens of Kepone. Administration of 15 mg/kg Kepone by oral gavage to Sprague-Dawley rats during gestational days 14 to 20 resulted in PVE in female offspring at 6 months of age (Gellert and Wilson, 1979). Abnormal cyclicity in adulthood, manifested as dose-related PVE, was also observed when rats received sc injections of 100, 250, and 375 mg/kg Kepone on postnatal day 4 (Sierra and Uphouse, 1986) or 0.2 and 1.0 mg/kg Kepone on postnatal days 2 and 3 (Gellert, 1978). Advanced vaginal opening in these females was also observed in both studies. Adult female Fisher rats administered 10 to 50 mg/kg Kepone i.p. on the morning of proestrus or diestrus exhibited PVE at the highest dose (Uphouse et al., 1984). Constant estrus was also observed in adult Balb/c mice fed 30 to 40 ppm of Kepone 1 to 2 months prior to and 3 months after mating (Huber, 1965).

HCB has been isolated in several species in the reproductive tract. High concentrations of ^{14}C-HCB have been isolated in the ovaries and egg yolk of

Japanese quail (Ingegrigtsen, 1985). Common terns of Hamilton Harbour have been found to contain 7.65 ± 3.57 ppm of HCB (Gilberston and Reynolds, 1972) while gannets from Norway have been reported to contain 0.033 ± 0.0018 ppm of HCB (Fimreite et al., 1982).

The adverse reproductive effects of HCB have been studied in rats, minks, ferrets, and monkeys. Grant et al. (1977) fed weanling Sprague-Dawley rats HCB in the diet for four successive generations. They observed decreased fertility at 320 and 640 ppm of HCB and decreased litter size and an increased incidence of still births at 160 and 320 ppm of HCB. No effect on fertility or fecundity was observed in weanling rats fed 60 to 140 ppm of HCB 96 days prior to mating and during two successive pregnancies in a study by Kitchin et al. (1982). Kheru (1974) and Simon et al. (1979) reported negative dominant lethal tests in the rat in doses of 20 to 221 mg/kg/day for 5 to 10 days.

Both minks and European ferrets exhibited reduced fecundity, manifested as decreased litter size and increased percentage of stillbirths following chronic feeding of 25 mg/kg of HCB prior to mating and during pregnancy. Ferrets were also anestrus during the normal breeding season in this study (Bleavins et al., 1984).

A study of Japanese quail fed 1, 5, 20, and 80 ppm of HCB in the diet for 90 days (Vos et al., 1971) indicated no effect on egg production or shell thickness, but a reduction in hatchability of eggs (53% to 100% at 80 ppm) and reduced egg volume at 20 and 80 ppm.

Iatropoulos et al. (1976) demonstrated that chronic feeding of HCB to rhesus monkeys induced dose-related reductions in oocytes. In addition, the animals appeared anovulatory. Despite these findings, the ovarian content of HCB was low compared with levels in the feet, bone marrow, and adrenal cortex (Knauf and Hobson, 1979).

In summary, although the data base is limited on the reproductive toxicity of Kepone and HCB in mammalian and avian species, the above studies provide some evidence of impaired reproductive function (measured by decreased fertility and fecundity or impaired cyclicity) following exposure to these pesticides.

Human Studies Studies for various pesticides in women are very limited. It has been shown that HCB is commonly present in fat due to its lipophilicity and tendency to bioaccumulate (Mes et al., 1982). Levels of HCB were determined in the blood of women who had suffered spontaneous abortions and women who had full-term deliveries (Leoni et al., 1986). The samples were taken immediately after the abortion or at the time of labor. HCB levels in the blood were 1.6 ± 1.6 ppb for those that aborted and 1.5 ± 1.1 ppb for the controls; this difference was not statistically significant. No mention was made regarding the fertility or reproduction of the people exposed to HCB in Turkey when thousands were exposed to high concentrations of HCB in their diet (Courtney, 1979).

HCB has been isolated in human ovarian follicle fluid in West Germany (Trapp et al., 1984) and Canada (Jarrell et al., 1988), although there have not been sufficient numbers studied to determine if such a presence has an effect on reproduction.

Occupational exposure to Kepone (chlordecone), a chlorinated hydrocarbon pesticide whose uses in the USA were cancelled April 11, 1977, induced toxicity, especially neurotoxicity, but no reports of female reproductive toxicity were reported by Epstein (1978), Cannon et al. (1978), and Barlow and Sullivan (1982).

Risk Assessment In the absence of sufficient human data, calculations were made on the available animal data to estimate safe exposure levels in humans, using the following EPA formulation, in which NOEL is no observable effect level, LOEL is the lowest observable effect level, Rfd is reference dose, and ADI is acceptable dose intake.

NOEL/LOEL = acceptable human exposure level
uncertainty factor = 100(Rfd or ADI)
(or 1,000 if no NOEL is available)

Using data on LOEL and NOEL when available for the above studies on Kepone and HCB, the ADI's listed in Table 6-9 were calculated.

For example, the most sensitive species exposed to Kepone is the mouse (Good et al., 1965) where the LOEL, using fertility/fecundity as an end point, is 5 ppm (no NOEL available); the ADI = 5 ppm = 5 x 10^{-3} ppm 1,000.

Future Studies

- ***Animal*** Future animal studies should include (1) additional well-designed studies in laboratory animals using protocols that adhere to risk assessment guidelines (e.g., appropriate dose levels, adequate exposure periods, collection of pharmacokinetic data); (2) interspecies comparative studies, including studies of the menstrual cycles of animals (e.g., nonhuman primates); and (3) additional studies to evaluate a broader

TABLE 6-9. Calculation of Acceptable Human Exposure Level (ADI) from Animal Studies

	Endpoints	
Compound	Fertility/Fecundity	Cyclicity
Kepone	5×10^{-3} ppm[a]	3.3×10^{-3} ppm[b] 4×10^{-3} mg/kg[c]
HCB	2×10^{-3} mg/kg[d]	2×10^{-3} mg/kg[e]

[a] Mice (Good et al., 1965).
[b] Mice (Huber, 1965).
[c] Rats (Gellert 1978).
[d] Minks/ferrets (Bleavins et al., 1984).
[e] Ferrets (Bleavins et al., 1984).

range of pesticides (e.g., representative compounds from different classes, families).

- ***Human*** Because the human data base is so limited, the following information is required to make adequate risk assessment calculations: (1) studies to determine environmental exposure levels of pesticides; and (2) appropriately designed epidemiologic studies that assess reproductive toxicity resulting from pesticide exposure.

MATHEMATICAL MODELS FOR REPRODUCTIVE TOXICOLOGY

Biophysical/physiologic modeling is potentially of great value in addressing two important problems fundamental to the assessment of toxic hazards:

- Extrapolation of dose-response curves obtained in laboratory studies (necessarily performed at high dose levels) to the comparatively low exposures of principal interest in risk assessment.

- Cross-species extrapolation; perhaps better phrased as cross-species dose-response estimation--that is, the scaling of quantitative dose-response data (obtained using laboratory animals) to humans.

Both problems have long been addressed by various intuitive, empirical, and even formal mathematical techniques. Scaling doses for body weight, surface area, or other criteria is common practice, notwithstanding documented counter-examples of "paradoxical" sensitivity (or insensitivity) of a particular species or an individual subject to a specific compound. The modeling work reviewed briefly here represents some of the more advanced attempts to provide a detailed, formal, explicit rationale for dose-response estimation.

Given the ethical and moral unacceptability of direct human experimentation, formal mathematical modeling has the potential to offer significantly improved dose-response estimation. However, many view the literature on the subject with healthy skepticism. It may appear to provide rather tenuous arguments upon which to revise prior estimates of toxicity. Assuming that prior estimates of toxicity for a putative hazard were properly biased toward safety (e.g., with a low estimate for the "virtually safe dose," VSD, from intentionally "conservative" extrapolation) it is understandable that more elaborate modeling would tend to produce higher VSD estimates. Also, understandably, this seeming methodologic bias toward relaxed exposure standards may provoke concern and criticism. Since by nature, properly applied formal methods also tend to make arguments explicit, it can be hoped that the debate will be focused and constructive.

Further broad reasons for concern with formal modeling are: (1) intuitive notions have a direct, simple, unbiased appearance such that methodologic analysis can seem superfluous, yet this may not be the case; and (2) alternative, seemingly complicated models can be so imposing as to stifle debate, yet may be shown to represent rather simple ideas. Methodologically, the important issues are (1) the selection and proper application of the best suited techniques; and (2) broad insight into methodologic limitations of these techniques and the manner and degree to which their limitations may affect public policy conclusions. Of course, the importance of selecting and properly applying the "best suited" techniques is an obvious, easily cited, but technically ill-defined objective that may not be readily achieved.

Probability and statistics are the mathematical subtopics most closely associated with modeling in pharmacology, toxicology, epidemiology, risk assessment and cost/benefit analysis; however, other mathematical subdisciplines may also be the source of useful ideas and techniques, an example given below being dynamical system theory. Survey of the topic can be undertaken in two ways: (1) by determining what techniques have been applied in toxicology or, specifically, to reproductive risk assessment; and (2) more abstractly, by preliminarily examining the medical and physiologic questions that require analysis to identify, if possible, fruitful mathematical analogies. This dichotomy, combined with the modeling issues identified earlier--appropriate selection and proper application and insight into limitations--suggests a fourfold approach to this review.

The study-group contractor performed a TOXLINE computer literature search using keywords as follows (with raw citation counts in parenthesis): models (yielding 64 items), risk and assessment (53 items), statistics (63 items), and epidemiology (63 items). The set intersection (i.e., overlap) was small. Unfortunately, this search yielded only two specifically relevant leads, and an independent effort was begun to examine the literature search problem itself. Clearly, despite the seemingly inclusive character of the search terms, the total list represented only a minute sample of the relevant literature.

Subsequent analysis indicates that reasons for the initial search failure include the following: (1) virtually all contemporary "scientific" research involves some mathematical/statistical analysis, but, by this very fact, authors and journals in the sciences may not choose to address mathematical methods explicitly in their titles or keywords; (2) there is subjectivity and considerable latitude permitted in the assignment of keywords; moreover, practices vary among journals; and (3) hundreds of journals in applied math are not systematically indexed in the TOXLINE data base, journals for which, in any event, the chosen keywords would have been inappropriate and inadequately delimiting. Further, there is a large, growing literature in such clearly related fields as the econometrics of environmental regulation that has not been investigated. Thus, the sample bibliography is neither comprehensive nor even properly representative, though it may be judged interesting, informative, and worth pursuing. As one would expect from the broad meaning and use of terms such as "model" and "risk assessment," the search flagged an assortment of articles of only tangential relevance to mathematical models for reproductive risk assessment. A new start has been attempted using two CD–ROM-based bibliographic search aids: "Compact Cambridge--MEDLINE CD" and "Silver Platter--CANCER LIT." An appendix contains preliminary search results using these tools (several hundred reasonably relevant citations). As in common experience, a more productive approach has been to scan the literature for interesting review articles from which secondary citations can be pursued. The actual bibliography of 20 odd items was compiled by this means.

The literature on formal modeling relevant to the current report can be classified into broad categories as follows:

1. Models of reproductive function
    - Mammalian
        - Female
        - Male
    - Nonmammalian

2. Biophysical models of toxic effects
    - Carcinogenic
    - Noncarcinogenic

3. Constrained statistical regression

The Category 1 models found in the literature search, thus far, will require considerable revision/extension to be useful for toxic hazard assessment. This is principally because the putative mechanism(s) of toxic action will require explicit specification in the context of existing model components (Ferin and Bogumil, 1988). Although possibly the most useful models in the long term, they are not considered in the present report.

Biophysical models of toxic effects appear to be undergoing quite active development. Currently, the most popular subclass in this category represents an elaboration of traditional models of tumorogenesis (placed for distinction in category 3 below) to reflect organism uptake and internal distribution and/or metabolism of the applied compound. In other work, noncarcinogenic toxic processes have also received some attention (e.g., in the context of electrolyte balance and hemoglobi n-0_2 binding).

Constrained statistical regression models may be judged to be intermediate (with overlap on both sides) between general statistical procedures and the somewhat more physiologically detailed models of category 2. Category 3 models provide a priori constraints on standard data-fitting/curve extrapolation procedures so as to reflect known or hypothesized biophysical factors (e.g., the number of "stages" in tumor development or the influence of "background" exposure).

Due to time and space limitations, the brief commentary below is limited to models in the categories carcinogenic and constrained statistical regression, perhaps the subset of most immediate interest; however, as noted above, these models were not intended to describe reproductive end points. Moreover, although they are intended as general models for tumorogenesis, they may not be directly applicable to sex-organ tumor incidence dose-response data for various reasons also reflected in epidemiologic studies.

The most widely applied carcinogenic and constrained statistical regression models are motivated by the notion that carcinogenic agents act by damage to the DNA of a single cell, requiring one or more molecular "hits." Secondarily, there may be one or more subsequent stages of tumor promotion that may be required for full expression. Background effects and the process of DNA "repair" have also been incorporated, providing a measure of reversibility. Carcinogenic models go further in attempting to account for internal distribution and/or metabolism, factors of possible importance in estimating the effective "target organ" dose as distinguished from the exposure or experimentally administered dose. These models have evolved over several decades, as briefly summarized below.

Work dating from the 1950s has been reviewed by Armitage and Doll (1961). At root, most current models of carcinogenesis derive from these pioneering efforts; what has changed more than the models is the regulatory thrust of current applications. Mantel and Bryan (1961) proposed a method to calculate an upper confidence limit for the incidence of tumors from the proportion observed at a particular test dose. They synthesized a dose-response curve, extrapolating towards zero incidence (e.g., 10^{-8}) to obtain an estimate for a VSD. Their extrapolation presumed a probit response line with a slope of unity, which was regarded as conservative because many observed dose-response regression lines are steeper. The method was later refined (Mantel et al., 1975) to allow for background tumor incidence and for extrapolation from experiments using multiple doses.

Subsequently, doubts were expressed about the wisdom of using probit models for extrapolation. Although a probit line with unit slope may appear "conservative" in the vicinity of the actual data, when extrapolated orders of magnitude closer to zero, the circumstance can change. This has been discussed by Mantel and others, both in the context of the "one-hit" model, which effectively assumes linear extrapolation, with response approximately proportional to dose and in relation to various alternatives (e.g., Chand and Hoel, 1974; Schneiderman, 1974; Hoel et al., 1975). One alternative model on which a great deal of work has been done is based on the multistage concept of tumor induction, supported both by animal experiments and from epidemiologic data (Peto, 1977). According to the basic version of this model, if rates of occurrence of, say, *m* changes are all directly proportional to the dose *(d)* of a carcinogen, the cumulative proportion (*P* as a function of dose) of animals with tumors after a fixed period of continuous dosage (and for values of *P* in the useful experimental range, e.g., ~10%) will be approximately of the form:

$$P(d) = 1 - \exp(-Bdm)$$

This assumes that when $d=0$, $p(d)=0$, i.e., that there is no background incidence. If we assume that the rate of occurrence of the i-th change is of the form a_i+B4_id (to provide for a nonzero incidence when $d=0$), we have a modified formula for $P(d)$:

$$P(d) = 1-\exp\left[-\prod_{i=1}^{k}(a_i + B_i)*d\right]$$

The properties of related models have been explored in detail (e.g., by Crump et al., 1976; Guess et al., 1977; Crump et al., 1977; Brown, 1978a), and a program for maximum likelihood regression is available (Crump et al., 1977). Depending on "best fit" parameter values to specific experiment data, the model response curve may be essentially linear at low dose--i.e., the extrapolated slope of $\log\{P(d)-P(0)\}$ versus log d is unity--or the slope may be significantly steeper. However, extrapolation is always problematic; even when the data yield best-fit parameters with a low-dose log-log slope as high as two or more, model parameter-interaction effects have been shown to leave open the possibility of nearly equivalent fits to the observation points that will lead to a low-dose log-log slope of unity. The model itself is not strongly predictive in this respect. Consequently, the usual recommendation is that a "lower limit" for the VSD be obtained by linear extrapolation.

In discussion of the low-dose behavior of the response curve, a question of interest is how the carcinogenic effect, if any, combines with any spontaneous or background level. One model, used by Mantel et al. (1975) and several other authors, assumes that the two causes act quite independently, by different biologic mechanisms, yielding approximately RMS additive tumor incidence. Another possibility is that the two causes affect the same mechanism, so that the test substance can be thought of as adding a certain amount to a virtual background dose, and the latter is essentially assumed in the multistage model. The important point is that, with nonzero background, the additive dose assumption implies low-dose linearity. Moreover, as Hoel (1980) has shown, if both mechanisms are present (e.g., because the background incidence arises from several causes) then low-dose linearity will be a good approximation under broad conditions. Notwithstanding the arguments for linear extrapolation as a means to ensure "conservative" VSD estimation, it has been remarked that the larger problem is one of decision--whether a substance should be banned and, if not, what the permitted upper limit of exposure should be in a cost-benefit tradeoff. For most substances under consideration, there are certainly benefits as well as risks. If there is a specific exposure level for which the net gain is maximized, the adoption of a more stringent standard on grounds of conservatism will lead to a loss of potential benefits that outweigh the reduced risk.

The possible importance of distinguishing between the administered dose and the "effective" dose reaching the site of action is self-evident. In the absence of other evidence, the standard assumption has been that one is directly proportional to the other. However, if the actual relationship is not linear across the scale from lab dose to environment, the default assumption will lead to erroneous dose-response extrapolations. Cornfield (1977) proposed a kinetic model involving two simultaneous reactions with the test substance: one with a free substrate to form an activated complex; the other with a free deactivator to form a deactivated complex. In many respects the published model was very poorly conceived; however, subsequent work has led to better reasoned models incorporating similar ideas. Van Ryzin and Rai (1980) have applied some adjustments for metabolic activation to data for vinyl chloride, providing a possible explanation for an unexpected high-end flattening of the dose-response curve, at about 15% to 20% response. Hoel et al. (1983) and Van Ryzin (1986), among others, have used similar models to reexamine the low-dose extrapolation problem. In broad terms, the conclusions have been that, for a number of substances of practical interest, nonlinear reaction kinetics play a significant role in determining the "target organ" dose. For such compounds, traditional models will yield biased VSD estimates. In the reported cases, VSD estimates have been revised upward, but this is not an automatic feature of the general model.

It has been proposed that models incorporating species-specific descriptions of relevant metabolic reactions (e.g., compound activation/detoxification, etc. as per above) could greatly improve the accuracy of cross-species dose-response estimation. Andersen et al. (1987) have attempted this for methylene chloride-induced tumors, concluding that human exposure, in terms of the effective "target organ" dose, may be less than 1% of the value that would be estimated by traditional extrapolation from high-dose mouse data. Their result is not critically dependent on species characteristics--the more significant consideration is the existence (in several species) of two metabolic pathways for the compound, one nontoxic but saturable; the other, producing the toxicant, becomes significant at high concentrations. However, as reported, detailed model validation in each comparison species was not undertaken. Some model parameters were estimated by allometric scaling across species. Generally, the possibility exists for significant species-specific differences in various metabolic rates, partition coefficients, and other critical model parameters, factors for which cross-species scaling may be no more certain than for the original problem. Thus, although the compartmental distribution and reaction-kinetics idea has great promise, it will require very cautious application.

The models discussed to this point do not consider the time at which individual tumors first appear during an experiment--potentially very useful information. Models incorporating time-to-tumor have been developed by Hartley and Sielken (1977, 1978), Rai and Van Ryzin (1979), and others. Application of these models has been hampered by the relative difficulty of the required protocol. Larger sample sizes, using standard models, may be more cost effective. The literature contains many papers in which various models have been fit to several sets of data and the low-dose extrapolations compared. The results are broadly in line with expectations: comparatively low VSD estimates are associated with the multistage model with higher values from the probit model; however, even this generalization need not be true for all compounds and dose-response curves (Krewski and Van Ryzin, 1981). Tumor incidence models incorporating metabolic/distributional kinetic subcomponents have been less widely applied, presumably due to the additional data needed to estimate reaction rates, distribution volumes, partition coefficients, and/or other factors.

METHODS FOR RISK ASSESSMENT IN REPRODUCTIVE TOXICOLOGY

The data presented in this chapter lends credence to the substantial and growing concern over the effects of pesticide exposures on reproduction. Clinicians in obstetrics and pediatrics, as well as other health scientists, are frequently confronted with questions concerning the effects of a drug or chemical on human reproduction or development. In some cases there is insufficient data to identify the drug or chemical as a reproductive toxicant. That fact, however, frequently derives from inadequate knowledge about experimental animal or human response following exposure (Table 6-10). For this reason, it is almost always necessary to extrapolate reproductive toxicology data across species to arrive at a prediction of the likelihood of human hazard and estimate the degree of human risk.

In spite of gaps in our knowledge of reproductive vulnerability to xenobiotics across species, data and theoretical approaches are available that would allow more rational prediction of human reproductive and developmental risk. The approaches to be utilized for risk assessment in reproductive toxicology include dose-response models, pharmacokinetic models, physiologically based pharmacokinetic models, and expert systems.

Dose-response models may be used to explore methods to extrapolate dose-response information across species. Because biologic information is hidden in dose-response models, the uncertainty associated with the extrapolations necessary to compute quantitative human risk is undefined. For

TABLE 6-10. Recommended Species for Evaluation of Reproductive Endpoints

Species	Male	Female
Mouse	Spermatogenesis Testicular pathogenesis Epididymol sperm Cellular biochemistry Fertility *In vitro* fertilization	Embryogenesis (cleavage) Ovarian pathogenesis Estrus cycle Endometrium
Rat	Spermatogenesis Testicular pathogenesis Hormone profiles Hormone challenge Epididymal sperm Cellular biochemistry	Embryogenesis (cleavage) Ovarian pathogenesis Estrus cycle Endometrium Fertility
Rabbit	Motility Ejaculatory function Sperm function Fertility Secondary sex organs Hormone profiles Artificial insemination *In vitro* fertilization	Oviduct Hormone profiles *In vitro* fertilization Embryogenesis (cleavage) Hormone challenge
Pig		Ovarian morphology Estrus cycle Fertility Hormone profile Hormone challenge *In vitro* fertilization Embryogenesis (cleavage)
Monkey		Menstrual cycle Oviduct Uterus Endometrium Fertility Hormone challenge Follicle development

this reason, additional biologically-based approaches for quantitative risk estimation should be explored.

Pharmacokinetic models contain more explicit biologic information than dose-response models. The explicit use of biologically relevant parameters such as volume of distribution and rate constants for transfer between compartments or elimination from a compartment provides parameters that can be scaled or extrapolated across species. Unfortunately, the biologic parameters, volume of distribution, and rate constants for transfer and elimination are complex formulations of organ volume, organ metabolic rates, organ blood flow, etc. Therefore, to increase the explicit consideration of all available biologic parameters, physiologically-based pharmacokinetic models should also be explored.

Physiologically based pharmacokinetic models contain a broader array of explicit biologically relevant information than do pharmacokinetic models. This increase in biologic relevance unfortunately carries a substantial price --much more experimental and clinical information is required. Using the physiologically based pharmacokinetic models, less extrapolation is required because species-specific data are included explicitly in the risk assessment model. These parameters may include organ volumes, blood flow to specific organs, and rate of xenobiotic clearance by specific organs.

Dose-response, pharmacokinetic, and physiologically-based pharmacokinetic models all use numerical information and algorithmic methods. Unfortunately, the process requires knowledge of species differences in reproductive biology, development, pharmacology and toxicology. Because of the complex nature of knowledge required to make a rational judgment of human risk, the utility of expert systems will also be explored for risk assessment in reproductive and developmental toxicology.

In addition to developing theoretical models for risk assessment, *in vivo* and *in vitro* experimental models are available in the laboratories of the investigators, which will allow testing and refinement of the data used for risk assessment and the risk assessment models on an ongoing basis.

Safety Factors

At the present time, risk assessment in reproductive toxicology is conducted by using safety factors--multiples of 0.1 used to adjust a defined dose or no-observed-effect-level in an experimental animal model to the permissible level for human exposure.

One example of recommended safety factors for developmental toxicants that utilizes this "factor of 0.1" approach has been proposed by Wilson (1973) and modified by Schardein. This approach is also proposed for reproductive and developmental toxicants in Proposition 65 (California Health and Welfare Agency, 1988). Although the safe level in this factor of 0.1 approach is defined by exposure or use, it is independent of species differences in uptake, distribution, metabolism, clearance, or sensitivity. Even worse, a safety factor approach to permissible human exposure levels to putative reproductive toxicants is doomed to overregulate xenobiotic compounds that are false positives and allows excessive human exposure to false negatives, thus increasing social costs and impairing human reproduction and health.

Conducting risk analysis using safety factors does provide a level for permissible human exposure; unfortunately, this number cannot be adjusted by advances in reproductive physiology, pharmacology, toxicology, or risk assessment. Using safety factors to set permissible human exposures is unacceptable, because the uncertainty of the estimate of risk is completely undefined. A more rational approach for risk assessment would be to determine the physiologic, pharmacologic, toxicologic, cellular, and molecular characteristics that control reproductive and developmental toxicity in experimental models and translate that information into predicted human risk based on human characteristics that are similar.

To use this approach, specific needs should be addressed:

- The first need is to gather data on reproductive effects of pesticides across species. Computerized data bases should be developed that encourage efficient use of previously collected information on reproductive physiology, pharmacology, and toxicology.

- The second need is to develop methods for translating information on reproductive hazards from pesticide treatment of experimental animals into risk assessments that protect human populations.

- Third is the need to continue to develop experimental and theoretical models that can define the site and mechanism of action of pesticides that are reproductive toxicants (NRC, 1985).

The specific research initiatives that will be emphasized to conduct and improve reproductive risk assessment are: (1) physiologic, pharmacologic, and toxicologic data bases appropriate for reproduction, (2) dose-response models, (3) pharmacokinetic models, (4) physiologically based pharmacokinetic models, (5) expert systems, and (6) ongoing coordinated research using *in* *vitro* and *in vivo* models to refine the data and methods used in quantitative risk assessment.

Data Base Development

One of the major limitations in conducting risk assessments in reproductive toxicology is the diversity of physiology, pharmacology, and toxicology across species. This suggests that for extrapolation across species, risk assessments require access to a broad range of data detailing species differences in reproductive physiology, pharmacology, and toxicology.

Models for Risk Assessment

Models of many types are used in all phases of biologic research (NRC, 1985). They are simplified rules or systems used to organize our view of biologic structure and function. In both an experimental and theoretical sense, models are used to define and predict the responses of complex organisms or organ-systems to external forces or factors. This portion of the report will explore models that can be used to predict human risk for adverse reproductive outcome following xenobiotic exposure.

Dose-Response Models Dose-response models are tools used to express the dose-response relationship observed in animal experimentation. These models involve exponential or polynomial equations of the general form,

$$Response = A \times Dose + B \times Dose^2 + \ldots$$

although other equations are also used (NRC, 1988; Rai and Van Ryzin, 1985).

These statistical models of the dose-response relationship contain substantial amounts of biologic information. Unfortunately, the biologically important information that is contained in dose-response models is hidden in a very complex fashion in the coefficients and exponents. Therefore, the coefficients and exponents of the dose-reponse models cannot be altered by increased information about biologic similarities and differences between species. This is one of the major limitations of dose-response models.

Pharmacokinetic Models Pharmacokinetic models are generally compartmental descriptions of the structure of interest. Although they are of value in many clinical situations, these models may be limited in defining the target tissue dose of a compound that is important in risk assessment. In addition, compartmental pharmacokinetic models do not provide a direct approach to account continuously for alterations in reproductive physiology.

As such, compartmental pharmacokinetic models represent static images of the system at a particular time or stage of development. This should not be interpreted as suggesting that pharmacokinetic models are of little value. Pharmacokinetic models can be and have been used to provide insight into the effects of hormonal alterations, placental function, physiologic changes during pregnancy, growth, and development on xenobiotic processing and toxicity (Mattison, 1986; Mattison and Jelovsek, 1987; Gillette, 1977; Slikker et al., 1982).

Physiologically Based Models Physiologically based pharmacokinetic models represent a different approach to the formulation of a quantitative model. This approach is appealing to many biologists because physiologic models retain biologic, physiologic, and anatomic information as discrete parameters that can be modified as appropriate for species, age, disease state, etc. The ability to modify the parameters of physiologically based models is especially appealing for biologists exploring reproductive processes with changing characteristics, including vulnerability. Physiologic models are also appealing to toxicologists because they allow a direct approach for the evaluation of target tissue toxicity (e.g., testis, ovary, placenta) and metabolic cooperation between organs (e.g., maternal liver, placenta, and fetal liver).

Physiologic models offer significant opportunity for extrapolation within and across species during the complex process involved in reproduction. In addition, physiologic models should allow appropriate extrapolation between species because parameters for comparable stages of gonadal function also will be utilized (e.g., gonadal volume, gonadal blood flow, gonadal metabolism). Similarly, species differences in accessory sex organ metabolism and blood flow can be incorporated into physiologic models for reproductive risk assessment.

Another attraction for the application of physiologic models to risk assessment in reproductive toxicology is that similar approaches are being developed for risk assessment in other nonreproductive end points, such as carcinogenesis. This means that all risk assessment procedures that use physiologically based models will be linked by the common denominator of biologic structure and function.

Expert Systems Expert systems provide another dimension to risk assessment beyond the numerical approach taken by statistical, pharmacokinetic, and physiologically based approaches (Fiskel, 1987). In addition to numerical data, expert systems can also include such qualitative heuristic information as the response of the species being studied to similar chemicals. Although the use of expert systems in risk assessment is new, some initial attempts have been made to develop expert systems for hazard identification in developmental toxicity (Mattison and Jelovsek, 1987).

In Vitro and *In Vivo* Models

A number of experimental approaches are available to aid in the validation of pharmacokinetic and physiologic models for risk assessment in reproductive toxicology. Most useful for risk assessment would be *in vivo* human testing, but this is usually not possible for obvious ethical and medical reasons. Thus, to decrease uncertainty in reproductive risk assessment, it will be necessary to develop *in vitro* and *in vivo* models that accurately reflect human reproductive processes.

CONCLUSIONS

Unlike other health effect end points that may be adversely affected by pesticide exposure, reproduction is a couple-dependent biologic process. All methods designed to assess reproductive risk *must* consider this dependence on two individuals in both animal and human studies. A slight decrement in a reproductive parameter in one member of a couple may be amplified and could cause infertility.

Although laboratory animals have successfully identified potential human reproductive hazards, the literature suggests that human males may be more sensitive than most common laboratory animals used in reproductive studies. It is not known whether this is true with respect to female reproductive end points. There is a general need for a more extensive, more precise data base, which will enable the development of models to extrapolate reproductive risks from animal to humans.

Although reproductive testing is currently a required part of the pesticide registration process under the Federal Insecticide, Fungicide and Rodenticide Act, much of the data generated for this are not available in the open literature. The lack of access to these data limits the development of quantitative risk assessment techniques for reproductive toxicity.

RECOMMENDATIONS

1. Animal and human studies should be redesigned to define explicitly the mechanisms of acute and chronic effects of pesticides on reproduction. Studies on reproductive effects should be conducted in several unrelated species of experimental animals and should include those (e.g., nonhuman

primates) whose anatomic and physiologic characteristics make them most likely to reflect human vulnerability.

2. The National Center for Health Statistics should generate, at both national and regional levels, annual birth-rate data stratified according to maternal age, race, parity, marital status and occupation.

3. Pesticide registration requirements should be modified to include a more explicit evaluation of gonadal and other reproductive effects. The following parameters should be added: (1) couple-time to pregnancy (litter); (2) male testicular spermatid head count and cauda epididymal sperm motility; and (3) female vaginal cytology (2×/day × 10d) and ovarian follicle number and morphometrics.

4. Studies on the potential effects of pesticides on reproduction in both humans (epidemiology) and experimental animals should include: (1) time to pregnancy; (2) menstrual cycle characteristics including vaginal cytology and markers for ovulation as a measure of ovarian function; and (3) measurements (either clinical techniques or standardized questions) of libido, impotence and reproductive behavior.

5. Several long-term recommendations concern the need to develop a variety of new or modified approaches to research in reproductive toxicology. These include: (1) the need to develop new techniques (e.g. flow cytometry) to evaluate the effects of pesticides on germ cells, including the evaluation of individual chromosomes in sperm and oocytes; (2) the need to more fully characterize hormone patterns in experimental animals and humans; (3) the need to develop techniques for monitoring the reserve population of oocytes in the ovary and germ cells in the testes; (4) the need to develop *in vitro* methods to assess the function of Sertoli and Leydig cells in the testes and Granulosa and Theca cells in the ovary; and (5) the need for to develop additional assays for field studies of early pregnancy loss.

6. Greater emphasis should be given to studies (e.g., questionnaires, analytical and clinical techniques, and reproductive outcomes) on unique human population with known exposures to pesticides.

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CHAPTER 7

DEVELOPMENTAL EFFECTS

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Pesticides are designed specifically to interfere with biologic systems. Although efforts are made to limit their degree of toxicity and exposure to nontarget organisms, this is not fully achievable. Pesticides share with most other chemicals--whether they be naturally occurring materials, drugs, or industrial products--the ability to interfere with prenatal development, postnatal development, or both.

As part of the registration process, pesticides are tested for developmental toxicity[2] in pregnant laboratory animals by means generally accepted and used throughout the developed world. In general, adverse effects on prenatal and postnatal development are observed in offspring of females exposed to pesticide levels high enough to produce signs of acute systemic toxicity in the mothers. Other classes of chemicals, known to be used at levels that interfere with prenatal or postnatal development, produce effects when vigorously tested by these standardized means. The assumption is made that pesticides could also disrupt the normal developmental sequences of the human embryo and fetus if exposures are high enough. Unfortunately, human

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[2] Developmental toxicity is the presence of one or more end points that may be used for evaluating the potential of an agent to cause one or more of the following four manifestations in the conceptus or offspring: death (prenatal or perinatal); structural abnormality; functional deficiency; altered rate of growth; or any combination of these events.

exposure to pesticides may occur at levels disruptive of maternal homeostasis[3], where the mother may recover, but where the conceptus may be irrevocably injured.

Relatively few agents are recognized as selective developmental toxicants[4] capable of interfering with development while sparing the mother. Such selective developmental toxicity is not reported for pesticides in use today but a full determination of whether or not it can be detected in the available test data merits further review. This might be because standard test procedures for chemicals under consideration as potentially useful agents have detected such selective toxicity and precluded their further commercial development.

The extent to which pesticide products may or may not be contributing to the incidence of developmentally-related problems in humans is unknown. The factors necessary for basing such estimation on data from studies in animals are in use, and they markedly enhance the plausibility of extrapolation of animal data to humans. Unfortunately, much additional information regarding not just these factors but others as well (e.g., maternal and fetal pharmacotoxicology) must be available before the basic mechanisms of abnormal development are sufficiently understood to allow remedial or protective measures for the adequate safety of humans during the very labile developmental stages of life. Significant gaps in available knowledge include adequate understanding of effects more subtle than those presently evaluated in the standard tests, understanding of the mechanisms of action that result in altered development, and last, but far from least, much more extensive evaluation and quantification of human exposure levels and the developmental consequences of exposure experienced by pregnant women.

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[3] Maternal toxicity is detection of an adverse effect upon the homeostasis of the mother during pregnancy. A more extended list of additional pertinent end points applicable to studies with laboratory animals has been produced by the EPA (EPA, 1986b).

[4] Selective developmental toxicants are agents that perturb development at exposure levels lower than those which overcome maternal homeostasis and which produce acute overt maternal toxicity.

INTRODUCTION

The purpose of this report is to examine the potential of pesticides to affect adversely the development of the human conceptus. The general paucity of data regarding potential hazards to the human conceptus necessitates, for the most part, evaluation of pertinent data obtained from studies in laboratory animals and extrapolation of the findings to humans.

From the spring of 1962, when the drug thalidomide appeared to be responsible for the increased incidence of a syndrome of malformations[5] detected in the newborn children of many countries (Lenz and Knapp, 1962; McBride, 1961), there was pressure to develop a test in animals that could detect abnormal morphologic development *in utero*. Such a test, devised by teratologists working in union with the Food and Drug Administration (FDA) and the World Health Organization (WHO), was circulated by the FDA in 1966. Subsequent application of this test for detection of *in utero* developmental toxicity has resulted in most of the pertinent data available for identification of this hazard among drugs and chemicals, including pesticides. Most of the published articles and reports of these studies have been collected and indexed by the Environmental Teratology[6] Information Center (ETIC), which was established in 1974 (Staples and Jordan, 1977). Bibliographic information from 1950 to 1988 is made available to the scientific and health community through the National Library of Medicine's online file ETICBACK, a component of TOXNET. The newer files are being established currently in a format similar to MEDLINE and will be called the Developmental and Reproductive Toxicology (DART) database.

Unfortunately, it was not generally (uniformly) recognized until the early 1970s that what had become known as the "teratogenicity test" really should have been named the "developmental toxicity test," because its design allows for production and detection of several developmental effects including gross structural abnormalities, even though it is not the ideal means for production of terata (malformations) (Johnson and Christian, 1984). Thus, it was important to emphasize the need to include data on endpoints in addition to malformations among fetuses; e.g., prenatal death, growth alterations, and developmental variations (Wilson, 1973) and to include data on adverse effects present in the dam (Staples, 1975). Most recently, awareness of the importance of evaluating postnatal manifestations resulting from exposures during development of the conceptus has also increased.

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[5] A malformation is permanent structural change that may adversely affect survival, development, or function (EPA, 1986b).

[6] Teratology is the field of science that addresses the causes, manifestations, mechanisms, and prevention of prenatally-initiated abnormal development.

Developmental toxicology is the field of science devoted to the study of adverse effects on the development of the conceptus. More specifically, developmental toxicity is the study of adverse effects on developing organisms that may result from exposure of either parent before conception, of the mother during prenatal development, or postnatally to the time of sexual maturation (EPA, 1986b). For mammals, developmental toxicity has traditionally and commonly included adverse effects initiated or evident during *in utero* development. In reality, some adverse effects on development that are initiated at conception may not be clinically evident until completion of morphologic and functional maturation at puberty. The degree to which longevity and quality of the aging process may or may not be influenced by adverse effects on development is an area that is largely unexplored. Developmental toxicology is distinct from "reproductive toxicology," which involves the study of adverse effects upon reproductive function of the adult; for example, these effects might interfere with gamete survival, the desire or ability to mate, the ability to initiate or maintain pregnancy, the birth process, or the ability to care for dependent offspring. Most, if not all, bioavailable chemicals have the capacity to interfere with the course of development if administered at a sufficiently high dose level under proper conditions. Therefore, hazard identification hinges far less, and perhaps not at all, on whether or not an adverse effect on the conceptus occurs from exposure to a given chemical than on the exposure level needed to perturb development under various specified conditions.

Many chemicals are not uniquely toxic to the conceptus, but also produce overt acute toxicity in the mothers. The mother may tolerate and recover from the exposure, but the embryo can be irrevocably diverted into a path of abnormal development. Thus far, a minority of chemicals have been demonstrated to be of a second class: they appear to have developmental phenomena as their most vulnerable "target" and are potentially the most problematic developmental toxicants[7], because embryos may be injured by such exposure without any adverse signs evident in the mother.

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[7] A developmental toxicant is an agent with the potential to interfere with the development of an individual while *in utero* or after birth.

To date, risk assessment and risk management of developmental hazards for both broad classes of developmental toxicants depend on the application of safety factors to the no-observed-adverse-effect level (NOAEL) from a standard animal study or calculation of a margin of safety (MOS) based on estimated human exposure. These approaches assume the presence of biologic thresholds for chemicals; that is, at some low level of exposure, there will be no increase in the risk of adverse developmental outcomes above background incidence.

RELATIONSHIP OF PESTICIDE EXPOSURE AND HUMAN DEVELOPMENT

Breadth of Exposure

The U.S. population is exposed to both naturally occurring and synthetic pesticides. At very low doses, we may be exposed to synthetic pesticides in ornamental flowers or foods, in intentional remedial or prophylactic home use, and as environmental contaminants. Individuals involved in accidents with pesticides in domestic, commercial or industrial settings can be exposed to amounts that may be hazardous to both mother and child. Although some of these hazardous exposures are due to misuse (Baker et al., 1978; Cannon et al., 1978), careful epidemiologic follow-up of pregnancy outcomes, and thus a cause and effect association of human developmental toxicity, is lacking.

Exposure Period

Development is an exceedingly complex and exacting process. Normal progression from the single-celled zygote to the multicellular and complex functioning adult requires the synchronization of many known and as yet unknown developmental events and sequences. For each tissue and organ to develop fully and function normally, cells not only must differentiate, but must also induce other cells to do so. Complex intra- and extracellular materials must be formed by rapidly dividing cells to achieve and maintain spatial orientation during directional migration to form and regulate the size and composition of organ fields that must in turn control the complex morphogenetic sequences called embryogenesis. Many developmental phenomena continue for years after birth, but the more critical morphologic stages are those of the first trimester of pregnancy. Because such a variety of events occur on different time lines in different organs and systems, it is not surprising that the degree and nature of sensitivity to toxic exposure, as well as its eventual manifestations, vary greatly with developmental stage. The effects of exposure to a developmental toxicant may occur across species and may be dependent not only on such factors as developmental stage or timing of the exposure, but also its intensity. Thus, the final outcome of developmental disturbances can be death, malformations, growth retardation, functional disorders, or any of these manifestations in combination (Wilson, 1977) produced by mechanisms that as yet are mostly unknown.

Damage to the gametes before conception usually is expressed as reduced fertility. In addition, no organs are formed during the first two weeks of human development. Therefore, toxic insult during this period has markedly less potential to produce frank congenital malformations, usually resulting in death or no permanent effects being detected. Some chemical classes having genotoxic potential may cause developmental toxicity after paternal exposure or maternal exposure during the preimplantation period (Spielmann and Vogel, 1989). The major period of organ formation is between two weeks to two months of intrauterine life, at which time the conceptus is in the most vulnerable period of its existence. The embryo is one-fourth inch long at three weeks, one-half inch long at five weeks, and one inch long at two months. During this time, it is a fragile group of cells and tissues consisting of microscopically thin sheets of tissue that are twisting, folding, and forming tiny pockets of cells that quickly develop the organs characteristic of adults. A major consequence of toxic exposure at this stage is death. But this is also the prime period of vulnerability to the initiation of abnormal developmental sequences leading to major birth defects.

During the third to ninth months of gestation, the major developmental event is growth, as the fetus increases in mass from 50 to 3,500 grams. Anatomic and physiologic maturation of the newly formed organs begins and progresses rapidly as the embryo/fetus/neonate increases in size and complexity. Some malformations can be initiated during this period (e.g., cleft palate, and reproductive organ alterations), because some organogenesis is still occurring. During this period and beyond, major effects of toxic insult to the fetus are growth retardation and functional deficits of organs or organ systems. Additional growth and maturation of some organ systems, such as the nervous, renal, and respiratory systems, continue during the early years of life. Thus, postnatal toxic exposure can have the same types of effects as exposure during the fetal period. These exposures can occur in a suckling infant via mother's milk as well as directly to the young individual. Exposure of the young may be markedly severe due to their unique dietary pattern, small size, and large fluid intake in comparison with adults.

Of the over 1,400 active pesticide ingredients tested so far, perhaps nearly half have been associated with some capacity to produce developmental effects in experimental animals when exposure occurred during the period of most obvious organ formation (Schardein, 1985). Data on exposure during fetal and postnatal periods are more limited. A problem with determinations such as these is that careful consideration of relevant exposure levels tends to be absent from test protocols. It is assumed that most chemicals will interfere with development at least at maternally toxic exposure levels, and pesticides would not be considered likely exceptions to this generalization. Developmental toxicity testing of pesticides began before the importance of dose or exposure level was fully understood; therefore, reports such as Schardein's tend to cite agents as "positive" or "negative" without consideration of whether or not testing occurred or whether or not developmental effects coexisted with maternally toxic exposure. Functional deficit studies, for example, are not yet required by the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) guidelines, but are being proposed. Available data have been reviewed by Chernoff (1987) and Kavlock et al. (1987). Behavioral and other types of functional alterations have been reported for several pesticides (Chernoff et al., 1979; Gray et al., 1986; Ostby et al., 1985). Excessive exposure to pesticides may be related to increased perinatal mortality in laboratory animals and, in at least one case, some deaths may have been related to functional cardiac disorders (Grabowski and Daston, 1983). Postnatal exposure to a pesticide via a nursing mother's milk has been related to induction of cataracts in rat pups (Chernoff et al., 1979; Gaines and Kimbrough, 1970).

As is the case for chemicals in general, it is apparent that the theoretical potential of pesticides to elicit the full array of unfavorable effects on development in laboratory animals has been tangibly demonstrated in animal experiments at various stages of development.

Magnitude of Developmental Problems in Humans

The magnitude of the problem of unfavorable reproductive outcomes in humans has been historically underrated by some and perhaps overrated by others. Nevertheless, this type of affliction, most fully perceived in humans at the outset of their lives, has tended to receive less attention both in terms of funds for research and in general public attention than have some problems more typical of adults, particularly older adults.

One in fourteen infants (7 percent) born alive has congenital malformations recognized at, or shortly after, birth (OTA, 1985), and the number increases with increasing age (Shepard et al., 1975). Seven percent of infants born at full-term have low birth weight (2.5 kg or less), and between 8 and 17 percent are born prematurely (OTA, 1985). This means that approximately 100,000 infants are born with severe birth defects in the United

States each year. An additional group of children of approximately equal size is identified later in life to also have developmental disabilities. Approximately 25% of recognized pregnancies end in spontaneous abortion. In 1986, birth defects were the fifth leading cause of potential years of life lost (CDC, 1988) and were responsible for 21 percent of all infant deaths. The Public Health Foundation reported in August 1987 that state health agencies spend more money by far on mental retardation and developmental disabilities than on any other chronic disease category.

Certainly, genetic disease is a factor in the statistics quoted above, but not an overwhelming one. Wilson (1973) estimated that only 20 to 35 percent of birth defects have a genetic component (i.e., chromosomal anomalies, mutational events). This figure is still regarded as a realistic estimate, even though nongenetic causes are being recognized as responsible for the majority of cases in such health effects areas as mental retardation.

Data presently available are inadequate to make a meaningful estimate of the degree to which pesticide exposure may or may not be responsible for developmental problems in humans, either as a primary or contributing cause. The animal data, which have proven reliable predictors of adverse effects of pharmaceutical agents, clearly indicate that the potential is there, provided exposures occur at least at or near the maternally toxic levels.

Surveillance Systems

Official interest in birth defects surveillance became evident in the 1960s (Edmonds et al., 1981). Review articles in which human and animal data are compared and contrasted for specific agents or classes of agents may serve as a form of surveillance, and in many cases specific methodologies in animal research have been designed with surveillance as a primary goal. During the 1950s, it became firmly established through detailed studies in animals that *in utero* development was vulnerable to diverse exogenous chemicals. Some general principles of abnormal development began to emerge so that experimental studies could be designed and executed more efficiently. As early as 1966, specific classes of effects resulting from *in utero* chemical exposure of animal embryos were known, and recommendations for required reproductive studies were discussed at length. The FDA Advisory Committee on Protocols for Safety Evaluations and its Panel on Reproductive Studies concerning Food Additives and Pesticide Residues stated that the three-generational reproductive study (Fitzhugh, 1968) was developed from concerns about the effects of maternal malnutrition on the fetus and offspring (FDA, 1970).

Testing in Animals The United States Environmental Protection Agency (EPA) published teratogenicity testing requirements in 1978 under FIFRA. It mandated how testing was to be conducted and reported and differed little from the FDA guidelines, except that exposure was to be initiated just before implantation and concluded the day before delivery (EPA, 1978a). In 1982, EPA specified the kinds of data and information required under FIFRA to support the registration of pesticides (EPA, 1982), reflecting guidelines proposed in 1978 (EPA, 1978a,b; EPA, 1984a). Similar regulations also went into effect through the EPA for chemicals under the regulatory control of the Toxic Substances Control Act (TSCA); the most recent version of this document was published in 1985 (EPA, 1985).

In 1986, the EPA published the procedures it would follow in evaluating potential developmental toxicity associated with human exposure to environmental toxicants (EPA, 1986b). In the same year, a screening test for developmental neurotoxicity, to include behavioral and neuropathologic analyses was proposed (EPA, 1986a,c; Francis, 1987). This was finalized into test rules in 1988 and 1989 (EPA, 1988, 1989a). Although it specifically concerned the glycol ether family of chemicals, it might be modified to apply to other chemicals suspected of having neurotoxic properties.

One *in vivo* screen is the Chernoff-Kavlock test in which rodents are exposed at or near the maternal maximum-tolerated-dose (MTD) throughout organogenesis (Chernoff and Kavlock, 1982). Since exposure to toxic agents usually results in reduced viability and growth retardation, these two parameters are the primary ones used. Although it is noted that thalidomide provides an important exception to the foregoing generalization, this test provides a reasonable indication of the most likely major outcomes of the larger, more definitive developmental toxicity tests in animals, and even though it is considered by some to produce a high incidence of false positives, it is a useful part of the total spectrum of nonhuman test data (Chernoff and Kavlock, 1982; Kavlock et al., 1987; Seidenberg and Becker, 1987).

Recently, the importance of postnatal functional assessment became recognized as an important part of developmental toxicity testing (e.g., Johnson et al., 1988). Such studies were not mandatory in the United States except in a few cases until recently when the EPA proposed developmental neurotoxicity testing for pesticides. Requirements are in place in other countries (Barlow, 1985; EEC, 1983; Tanimura, 1985; WHO, 1986). Postnatal functional deficits, including behavioral alterations, constitute one of the four major manifestations of developmental toxicity (Jensh, 1983; Riley and Vorhees, 1986; Wilson, 1973). Numerous articles have been published supporting the use of behavioral testing as part of developmental toxicity testing (Butcher et al., 1980; Vorhees, 1983) but this is still a less than fully resolved issue (Koeter, 1988). Of concern is test validation and selection of tests from animal models to predict human functional deficits (Hutchings, 1985; Jensh, 1986; Rosa, 1983) and extrapolation of animal test results to humans (EPA, in press). A major limitation is that an almost infinite number of functional parameters (e.g., cardiac, respiratory, hepatic, immune) could be included in evaluation of functional deficiencies. Whereas it is important to know if an agent can or cannot produce a particular type of functional change, formalized use of behavioral tests merits caution, in part pending consistent ability to flag agents that are not identified by more standard test methods.

A number of approaches have been used to study the behavioral and other functional expressions of abnormal development (Grabowski and Daston, 1983; Johnson, 1989; Mactutus and Tilson, 1986; Spyker, 1975) and interlaboratory studies have been completed in some instances (Kimmel and Buelke-Sam, 1985). The major effort was the Collaborative Behavioral Teratology Study (CBTS) reported in 1985 (Buelke-Sam et al., 1985; Kimmel and Buelke-Sam, 1985). Results from this vigorous study indicated that there may be specific instances in which behavioral testing would be appropriate even though a number of methodologic and interpretive difficulties require caution in establishing regulatory guidelines (Steinberg, 1987; Zbinden, 1981). Risk assessment guidelines published by the EPA (EPA, 1982) include the use of postnatal functional data as part of the evaluation process.

In vitro systems using animal cells in culture from humans or lower animals can serve as the first tier level for screening agents in an effort to prioritize them according to their need for further developmental toxicity testing. Such rapid, inexpensive tests are necessary, as the number of chemicals already in commerce and the rate at which new ones are entering the marketplace exceed the capacity for pregnant animal testing by standard means. The more common means of testing require large numbers of animals and more facilities than are available. A use for test-tube-type tests would be to focus these resources on those chemicals and exposures most likely to be potentially hazardous to humans. These systems are useful as part of a tiered approach to developmental toxicity testing (Johnson, 1987) and, when coupled with consideration of exposure levels (Johnson, 1989), they may be a marked asset in the design of epidemiologic studies to detect situations of human developmental toxicity. *In vitro* systems can also be applied to elucidate (Kimmel, 1989) structure activity relationships, to analyze complex mixtures, and to detect and analyze mechanisms of action (Newman et al., 1989).

Multigeneration studies may be used as part of the surveillance process for testing compounds likely to be involved in chronic human exposures

(Collins, 1978; Fitzhugh, 1968). Two generation tests are required in the EPA requirements for testing pesticides for reproductive effects.

Studies of Human Populations. The Centers for Disease Control (CDC) is an agency of the U.S. Public Health Service, and its Birth Defects Monitoring Program (BDMP) is the first U.S. nationwide monitoring system for human malformations. Since its inception in 1974, it has used the resources of the Commission on Professional and Hospital Activities (CPHA), and approximately 1,200 hospitals have volunteered to participate. It is the largest single source of this type of data in the United States (Edmonds et al., 1981). Over 150 types of birth defects are systematically monitored, and analyses are completed historically with changes detected by comparison to base years. The BDMP monitors over one million births per year throughout the United States (about one-third of the children born in hospitals in the U.S.). The Metropolitan Atlanta Congenital Defects Program (MACDP), a comprehensive system used to identify birth defects in children born in the Atlanta, Georgia, area, is also conducted by the CDC. The MACDP was started in 1967 in collaboration with the Emory University School of Medicine and the Georgia Mental Health Institute (LeMarchand et al., 1986). It monitors five counties covering 1,750,000 people and 24,000 births per year at 20 hospitals.

The FDA, through its Epidemiology and Surveillance Division Center for Drugs and Biologics, is concerned with and participates in a number of surveillance programs including the Michigan Medicaid program, the FDA-Boston University Collaborative Drug Surveillance Program, the FDA-University of California Child Development Study, the FDA Cooperative-Boston University Slone Epidemiology Unit Birth Defect Study, and the FDA cooperative study with Yale University (Rosa, 1987). These programs have monitored over 100,000 pregnancies linked to over 6,000 suspected or confirmed birth defects.

In 1967, there was only one state surveillance program in effect; by 1977, there were seven (Flynt et al., 1987). As of April 1987, almost three-quarters of the states had developed or were developing adverse reproductive outcome surveillance systems; only 15 states were not developing any system. Three states had more than one system, 17 had programs in the development stage at various planning and implementing levels, 2 were conducting pilot programs, 1 would be initiating a program by 1988. There is considerable variability among the states in the type of data collected and, as a generalization, each functions with scant data regarding levels of exposure.

Maryland, Massachusetts, Florida, and California are four examples of state programs currently in operation. Maryland has operated the Maryland Birth Defects Reporting and Information System (MBDRIS) since 1983 (Khoury et al., 1986). The two state-related programs in Massachusetts are the Birth Defects Study of the Drug Epidemiology Unit, Boston University School of Public Health, which monitors 175 hospitals, and the High Risk Infant Identification System, operated by the Massachusetts Department of Public Health. A surveillance program is being conducted in Massachusetts at Boston Hospital for Women, Lying-In Division, a part of Brigham and Women's Hospital (Holmes et al., 1981; Nelson and Holmes, 1987). Florida has three programs currently in effect: the Congenital Defects Epidemiological Surveillance Program, the Congenital Defects Surveillance Project, and the Infant Metabolic Screening Program (Flynt et al., 1987). California has the largest population-based birth defects monitoring program in the world, the California Birth Defects Monitoring Program. The program was created by the state in 1982 and began operations in 1983. As of January 1987, 55 percent of all California births had been recorded and analyzed for about 500 birth defect conditions (CBDMP, 1987).

The wide variety of state programs currently in effect indicates a growing interest in birth defects monitoring systems. A regular forum is needed for states to share information, and additional funding and technical assistance are needed. There is a need for a national agenda to develop and implement surveillance programs involving interested state and Federal officials. Surveillance systems do have limitations, since data are gathered *a posteriori*; therefore, we should not be totally dependent on them as detection systems for isolating incidents related to exposure to specific pesticides at this time. However, active efforts should be made to further refine current surveillance methodologies. In addition, to be maximally effective, these systems also must include parental occupation to facilitate later, more detailed analysis of possible levels of exposure in follow-up studies. Specific populations at risk, such as migrant farm workers, should be identified, and surveillance programs should be established to monitor these populations, particularly when they are known to be exposed significantly to individual pesticides or groups of pesticides. It is of particular importance in such cases for states to develop and implement methods for interaction and information exchange concerning those populations that move among states. Continued monitoring through well-designed human surveillance programs is essential. These programs will provide important assurance for the continued health and welfare of the human population after exposure occurs.

PREDICTABILITY OF ANIMAL TESTS

Testing in pregnant animals prior to human exposure is the best available means for precluding adverse effects on human development. Testing for developmental effects expanded markedly after the thalidomide disaster, and the systems presently in place are a means for detecting potential hazards and risks. In some areas of toxicology, animals may be less than adequate human surrogates, but for developmental effects they have, at least retrospectively, shown adverse *in utero* effects from all but a few of the chemical exposures known to have produced abnormal human development. However, development is a complex sequence of events, and the tests can be confounded by numerous factors.

Although the presence of chemicals and drugs has been shown to play a role in the incidence of certain anomalies, the evidence supports the conclusion that exposure to presently acceptable environmental levels for chemicals is not an observed threat to human development. This conclusion holds true as long as one does not expose a pregnant woman to toxic levels of such agents, or as long as a significantly high safety factor is applied in the few instances where developing embryos have been shown to be more susceptible than their mothers to the toxic effects of an agent. Thus, as Warburton (1987) has pointed out, "physicians and the public should be taught that some degree of reproductive loss is normal and that the best insurance against being childless may be not to allot too short a period of childbearing."

The Nature of Developmental Biology

Development of the mammalian conceptus through the embryonic, fetal, and perinatal stages to the attainment of normal size, structure, and function is a marvel that we are only beginning to understand. Embryogenesis is recorded to have been studied for at least two millennia, and studies of what could be considered as modern, mechanistic developmental biology have a history of at least three centuries. During progressive ontogenesis, a wide diversity of cellular functions must be achieved and integrated in a closely regulated temporal sequence. One of the important cellular functions critical to the maintenance of normal development is cellular proliferation. Kohler et al. (1972) demonstrated that the mouse embryo consists of approximately 100 cells on day 8 of gestation and about 90,000 cells on day 10, just 48 hours later. If each cell in the embryo on day 8 were dividing, then each would have to divide ten times to attain this increase of cell number. That would leave but 5 hours for the cell to proceed through the cell division cycle, an extraordinarily short time for eucaryotic cells and shorter than published times for most, if not all, cancer cells. Not only must these embryonic cells proliferate very rapidly, but they must simultaneously form the primordia of the definitive skeleton and all visceral organs typical of the adult. To do this, embryonic cells carry out a wide diversity of functions, such as cellular migration, secretion, adhesion, segregation, induction, and differentiation. Interference with any of these processes can theoretically lead to one or more congenital malformations. Understanding of developmental sequences has slowly but steadily improved. For example, we have come to appreciate how some embryonic cells must die in an appropriate location at the appropriate developmental stage, and, if they do not, abnormal development may ensue (Milaire, 1976).

Genetic Modulation

The developmental toxicity outcome following exposure to a hazardous agent is under genetic control. This has been inferred from species differences in response to such toxicants as thalidomide. The genetic differences responsible for varying outcomes can reside in the maternal or embro/fetal genome. Chernoff (1980) has published a particularly clear example in which control of the developmental toxicity response resides in the maternal genome. Using a diallele cross-mating design, he studied three inbred mouse strains and found that the developmental toxicity outcome after exposure to ethanol relied on maternal genotype. Changing the genotype of the conceptus was inconsequential. Chernoff further showed that the high blood alcohol of the sensitive strain was due to a low hepatic concentration of alcohol dehydrogenase, the main enzyme of ethanol catabolism. Conversely, the most resistant strain had the highest level of hepatic alcohol dehydrogenase and the lowest blood alcohol concentration. These data are consistent with the idea that such maternal metabolic processes as protein binding may regulate the amount of toxicant available to the embryo and thereby control developmental toxicity outcome. This same enzyme, alcohol dehydrogenase, is important in the mediation of other environmental teratogens such as the glycol ethers (Ritter et al., 1985) and industrial alcohols (Ritter et al., 1987).

An example of developmental toxicity outcome dependent on the genotype of the conceptus is the teratogenic response to acetazolamide. This drug induces missing digits in the offspring of pregnant rats or mice (Layton and Hallesy, 1965), and these missing elements are curiously localized to the postaxial side of the right forelimb. Evidence pointing toward embryonic genotype control of teratogenic outcome includes the demonstration by Biddle (1975) using reciprocal crosses of inbred mouse strains sensitive and resistant to acetazolamide teratogenesis, which indicated that sensitivity is a property of the embryo and is not maternally mediated. Furthermore, embryo transfer experiments between sensitive and resistant mothers confirm the embryo as the site of teratogenic action (Biddle, 1975).

Timing of Exposure

Another critical factor which governs embryotoxic outcome after hazard exposure is the developmental stage of the conceptus at the time of exposure. Traditionally, prenatal development is divided into three somewhat discrete periods--preimplantation, organogenesis, and fetogenesis--and the usual adverse outcomes of hazard exposure during these gestational stages are death, malformation and developmental (growth) retardation, respectively. Exceptions to this generalization have been clearly documented, but more often than not, the type of outcome is heavily dependent on the developmental stage at the time of hazard exposure. This dependence is based on sound biologic information, which is why the type of outcome can often be predicted based on the timing of exposure. Thus, malformation from preimplantation exposure is not likely to occur, due to the fact that embryonic cells are similar to one another at this stage and retain the capacity to differentiate in many directions. Low exposure, even if it seriously affects few cells, may allow a sufficient population of multipotent cells to remain, which can compensate for a deficit and result in a normal offspring at birth. High exposure at this stage leads to deleterious effects in all embryonic cells, and the conceptus dies. Once organ formation begins, the conceptus becomes highly susceptible to the induction of structural malformations. This is no doubt due to the fact that discrete organs are forming, each relying on a variety of organ specific cellular functions in order to complete its formation. Affecting these processes can logically be expected to end in malformation, and experience attests to the veracity of this explanation. Death also can be induced during organogenesis if the maternal treatment level is sufficiently severe. Furthermore, *in utero* growth retardation is known to occur in some instances of exposure during the period of rapid organogenesis, either with or without production of frank, gross anatomical abnormalities. When all organs are formed and the conceptus reaches the fetal stage, it is no longer possible to induce gross developmental malformations. However, growth of these organ systems and functional maturity of these organ systems does take place during this period and thus it is these end points that should be affected by exposure at this stage. Growth retardation is also a common occurrence following exposure during the fetal period. Functional abnormalities are developmental toxicity outcomes that have received attention relatively recently. Therefore, at present, we lack sufficient experience to confirm whether the induction of such embryotoxic outcomes will follow present expectations, or whether additional experience will change our understanding of the biologic basis of functional abnormalities.

A number of other factors can influence gestational outcome. Many of these factors involve dosage in the broadest sense and presumably affect outcome by modifying the quantity of active agent that reaches the conceptus. Thus, the amount of agent administered, the route by which it is given, the frequency and duration of dosage, and/or the physico-chemical nature of the agent itself may be contributing factors in determining the developmental toxicity response.

Dose-Response Relationships

Developmental toxicity outcome, as with other pharmacologic and toxicologic effects, usually exhibits dose dependency. That is, the higher the exposure, the more frequent or more severe is the outcome. Neubert and Barrach (1977) described some of the peculiarities associated uniquely with dose-response relationships in developmental toxicity studies.

The distinct phase specificity shown by embryotoxic chemicals can confound dose-response relationships in a number of ways, especially when the test substance is given subacutely, as is the case in most safety evaluation studies. Organ systems are differentially sensitive to dysmorphogenesis at various developmental stages; drug transfer to the conceptus may vary depending on gestational stage; and a drug or chemical may be capable of inducing or inhibiting its own metabolism when given for more than a few days. These factors, if not sufficiently controlled, can confound dose-response relationships. Another peculiarity of dose-response relationships in teratogenesis concerns the upper limit on the frequency of a specific malformation due to the development of other defects in the same organ or system. Neubert and Barrach (1977) used the example of polydactyly induced by 6-mercaptopurine to illustrate this point. At low dosage, polydactyly was induced at moderately high frequency. As dosage is increased, more severe limb malformations are induced, including missing digits and even absent limbs, thereby preventing the possibility of developing polydactylous limbs. The same situation can apply to the dose-response relationship for total incidence of malformed fetuses when higher doses cause prenatal death. Thus, although suitable dose-response curves can be established in developmental toxicity studies, a number of potentially confounding factors must be borne in mind.

Perhaps a more important practical question is whether there is a threshold below which most drugs or chemicals will not induce developmental toxicity. Practical experience and intuition generally suggest that this is a valid assumption. Many published studies indicate that at low dosage the rate of growth, malformation, or death is not different from that in untreated animals.

In addition, we know that the mammalian conceptus has a great capacity to recover from serious insult, suggesting that the "single hit" idea of nonthreshold response appears to be an unlikely explanation for developmental toxicity outcome for the majority of cases. Proof that a threshold exists is an impractical goal mathematically, but many biologic difficulties also prevent this vast experience from being translated into a valid concept. First and foremost is the small number of animals used in most of these experiments. Typically, 20 to 25 pregnant rodents and 12 to 15 pregnant rabbits are used per group, which reduces confidence in the apparent threshold demonstrated. Secondly, spontaneous malformations and deaths are difficult to distinguish from drug-induced embryotoxicity. Generally speaking, there are only statistical approaches to determine whether the frequency of these outcomes is within control limits. Much more preferable would be a biologic method to determine whether individual occurrences are spontaneous or drug-related.

Stochastic Considerations

The processes of reproduction and development are so complex that it is not surprising that errors occur as a result of chance and without the assistance of recognized external causal factors. Some reports (Diamond, 1987; Roberts and Lowe, 1975; Shepard and Fantel, 1979) have estimated that up to 80 percent of human conceptuses fail to reach term; over 90 percent of these losses occur subclinically without the awareness of the mother (Edmonds, et al., 1982). Thus, there is some indication that developmental failures are more common than developmental successes.

More than 10 years ago, Wilson (1977) estimated that drugs and environmental chemicals (as independently acting agents) could be clearly established as the cause of only 4 to 5 percent of the observed developmental defects. Since then, it has not been possible to reduce the "cause unknown" percentage (65 to 70 percent) for such defects, but further resolution of the impact of environmental agents on human *in utero* development may follow from more contemporary and combined considerations of agent nature and exposure severity (Johnson, 1989). As Brent (1987) recently pointed out: "It is quite clear that the solution to birth defects problems will not occur even if all known and future environmental 'teratogens' were eliminated. This is due to the fact that only a small percentage of human malformations are due to environmental agents." In this and other publications (Brent, 1976), this investigator pointed out that the occurrence of spontaneous errors of development, or "biologic errors inherent in the reproductive and developmental process, similar to the concept of spontaneous mutations," is another explanation for the etiology of human malformations.

Chromosomal Imbalance

Although categorized separately from chromosomal imbalance, as many as 10 percent of human live births may have early- or late-onset genetic diseases (Brent, 1976). Particularly when early embryonic loss is considered, the bulk of common birth defects clearly have significant genetic components (Yager, 1973).

Abnormalities of chromosome number and chromosomal imbalance have been suggested to be contributing factors to the estimated high rate of fetal wastage (Wramsby, et al., 1987). However, with the exception of maternal age, almost no genetic or environmental factors are known to alter the rates of human chromosomal abnormalities (Warburton, 1987). Interestingly, studies on at least one genetic disease, insulin-dependent *diabetes mellitus*, have led to the hypothesis that the persistence of the high frequency of genes for diabetes in the general population is the result of a prenatal selective advantage resulting in preferential birth of offspring predisposed to diabetes (Vadheim, et al., 1986). It also has been suggested (Diamond, 1987) that a recent study (Dronamraju, et al., 1984) provided evidence that a prenatal advantage may exist for fetuses carrying a gene encoding cleft lip and palate. These are but two of several examples of how evolution may favor genes that penalize homozygotes with certain lifestyles, because those genes benefit heterozygotes (Rotter and Diamond, 1987).

Evaluating Animal Studies

Guidelines for evaluating data from laboratory animal studies in developmental toxicology have been published by the EPA (1986b). This document will not attempt to provide guidelines or discuss particular study types, but instead will focus on issues of importance for evaluating animal studies and for comparing the results of studies in animals with experience in humans. First, it is important to realize that adversely affected concepti appear in every study conducted according to the guideline referred to above. The adverse effects will appear in every dose group and in the control group. They will consist of a variable incidence of early death among some concepti and of major and minor structural alterations and developmental delay among others. Some are an expression of "developmental background noise" (spontaneously occurring effects not associated with the agent being tested), others may be due to interference in the maternal homeostatic condition by the test agent or its administration (Khera, 1985), and some may be due to the effects of the test agent upon the conceptus. Second, it is expected that virtually all chemicals, and all of those that are bioabsorbed, have the potential to disrupt the developmental process (Karnofsky, 1965). Hence, the results of each study have to be evaluated to ascertain whether the adverse developmental effects seen can be demonstrated to be associated with the agent tested. The next step, to determine with what degree of reliability the results of animal studies using the same test agent can be used to predict what is likely to happen upon exposure of the human conceptus/(extrapolation), is a difficult question.

In experimental animal studies, questions are often raised about whether a fetal effect on the conceptus is direct or indirect via effects on the placental membranes and secondary to maternal toxicity or physiologic changes. Since the mechanism for a given teratogenic outcome has never been completely elucidated, it would be impossible to assume that a malformation was the result solely of a direct fetal effect, except perhaps in the case of a genetic alteration. However, even then, the influence of the maternal environment may be great enough to alter the final expression of the defect. On the other hand, reproducible fetal effects occurring at maternally toxic doses should not be assumed to be secondary to maternal toxicity. For example, salicylates produce a number of teratogenic effects that are highly reproducible in rats depending on dose and time of exposure (Kimmel et al., 1971). The dose of salicylate required for teratogenicity usually causes some signs of toxicity to the maternal animal, but McGarrity et al. (1981) and Greenaway et al. (1982) have shown that a number of malformations similar to those produced *in vivo* may result from *in vitro* culture of the embryo-placental unit at concentrations found in maternal serum. Thus, the relationship between maternal and fetal effects is not clearcut, and end points should not be attributed to direct or indirect action, or a combination of both, without further study. Reproducible major malformations or other fetal toxicities that occur only at maternally toxic dose levels should not be ignored if such toxic exposure may occur occupationally, accidentally, or voluntarily (e.g., by alcohol consumption, cigarette smoking, or drug abuse). However, they may be of less concern at lower levels of exposure because the safety factor or margin of safety selected to protect the adult will also protect the conceptus (Johnson, 1988).

The large number of developmental mechanisms whereby normal development may be altered (Wilson, 1973) is indicative of the complex nature of the developing organism and the spectrum of biologic processes that can be disrupted during ontogeny. The problems that such complexity poses for predicting the effects of a chemical and the fact that no mechanisms in this area have been delineated clearly were discussed by Skalko (1981). It is important, however, to remember that as difficult as this area may be to work in, such studies are vital to improve our understanding and, therefore, the predictability of end points in animal models.

The issue of exposure is a critical one in developmental toxicology. Dose-response data may aid in ascertaining the extent of maternal toxicity, which then can be compared with the embryo effect levels. This information provides a reference for evaluating the relationship between developmental toxicity and general maternal toxicity. Dose-response studies also provide information for characterizing the lower end of the dose-response curve and identifying the no-observed-adverse-effect-level (NOAEL) for maternal and developmental toxicity. This information may be used to identify which chemicals are likely to be selective developmental hazards, for example, by the calculation of the ratio of adult:developmental NOELS (A:D ratio) (Johnson, 1987). However, it is important to recognize that agents lacking selective developmental toxicity can produce developmental effects should exposure occur close to the maternally toxic level.

There are a number of factors which may affect the expression of the dose-response curve, including: (1) power of the study design; (2) potency of the developmental toxicant; (3) types and combinations of end points; (4) developmental stage in which exposure occurred; (5) degree of placental transfer; (6) maternal pharmacokinetics; (7) conceptual pharmacokinetics; and (8) maintenance of maternal homeostasis. Therefore, dose-response data must be interpreted with care, taking these factors into account. Documentation of exposure and its relationship to the end points affected is extremely important, but is not often done in developmental toxicity studies. This kind of information can be very useful, both for determining the pharmacokinetic parameters of greatest importance in predicting outcome (Kimmel and Young, 1983), and for determining the shape of the dose-response curve for low-dose extrapolation.

In the assessment of developmental toxicity data, statistical considerations require special attention. Statistical analyses should be based on incidence per litter for each end point. The analytical procedures used and the results, as well as an indication of the variance for each end point, should be clearly indicated in the presentation of data. Analysis of variance (ANOVA) techniques, with litter nested within dose in the model, take the litter variable into account but allow use of individual offspring data and an evaluation of both within and between litter variance as well as dose effects. Nonparametric and categorical procedures have also been used widely for binomial or incidence data. In addition, tests for dose-response trends can be applied. Although a single statistical approach has not been agreed upon, a number of factors important in the analysis of developmental toxicity data have been discussed (Haseman and Kupper, 1979; Kimmel et al., 1986).

Studies that employ a replicate experimental design (e.g., two or three replicates with 10 litters per dose per replicate, rather than a single experiment with 20 to 30 litters per dose group) allow for broader interpretation of study results since the variability between replicates can be accounted for using ANOVA techniques. Replication of effects due to a given agent within a study, as well as among studies or laboratories, provides added strength in the use of data for the estimation of risk.

An important factor to determine in evaluating data is the power of a study (i.e., the probability that a study will demonstrate a true effect), which is influenced by the sample size used in the study, the background incidence in the end point observed, the variability of incidence in the end point, and the method of analysis. As an example, Nelson and Holson (1978) reported that the number of litters needed to detect a 5 or 10 percent change was dramatically lower for fetal weight (a continuous variable with low variability) than for resorptions (a binomial response with high variability). With most regulatory testing protocols currently recommending 20 pregnant rodents per dose group, it is possible to detect an increased incidence of malformations in the range of only 5 to 12 times above control levels, an increase in *in utero* death rate 3 to 6 times above controls, and a decrease in fetal weight 0.15 to 0.25 times that of controls. Thus, even within the same study, the ability to detect a change in fetal weight is much greater than for the other end points measured. Consequently, for statistical reasons only, changes in fetal weight are often observable at doses below those producing other signs of developmental toxicity. Any risk assessment should present the detection sensitivity for the study design used and the end point(s) evaluated.

Although statistical analyses are important in determining the effects of a particular agent, the biologic significance of data should not be overlooked. For example, with the number of end points that can be observed in developmental toxicity studies, a few statistically significant differences may occur by chance. On the other hand, apparent trends in dose-response may be biologically relevant even though statistical analyses do not indicate a significant effect. This may be especially true for the incidence of malformations or *in utero* death, where a relatively large difference from the control value is required to attain statistical significance.

EVALUATING HUMAN EPIDEMIOLOGIC STUDIES

A few critical reviews have been written on the comparability of animal and human developmental toxicity data and the reliability of animal data for predicting hazards for human development. Among these are Hemminki and

Vineis (1985), Kimmel et al. (1984), and Nisbet and Karch (1983). Each of these reviews has suggested that comparisons between experimental animal data and human epidemiology data should only be made with an understanding that there are a number of differences in the way in which the data are collected, analyzed, and interpreted. In particular, the precision with which a cause and effect relationship can be established and with which confounding variables can be controlled are very different for the two disciplines.

Guidelines for evaluating epidemiologic studies have been attempted (IRLG, 1981), and questions related to the evaluation of both experimental and epidemiologic data for the purpose of risk assessment (EPA, 1986b; Kimmel et al., 1986) have been addressed. The discussions in this section are drawn from these reports and the critical reviews cited above. In particular, a number of basic differences are pointed out between epidemiologic and laboratory animal research studies and their interpretation. These differences must be recognized and taken into account in any evaluation of the predictability of developmental effects from experimental animals to humans.

Basic Epidemiologic Concepts in Developmental Toxicology

Epidemiology is, because of its observational nature, an inexact science. Conclusions drawn from epidemiologic situations cannot be taken at face value, but must be examined to determine to what extent alternative hypotheses can be ruled out. Alternative hypotheses include, but are not necessarily limited to the following:

(1) Chance: Are the results statistically significant? Was the study power great enough to justify reliance on a conclusion of "no observable adverse effect?"

(2) Artifact: Was the population studied atypical in a manner which could affect the results? Were the end points measured accurately, completely, and identically for all study subjects? How accurate was measurement or estimation of exposure, and was it measured similarly for all subjects? Were important differences in groups (e.g., groups of exposed and unexposed individuals) accounted for in the study design and analysis?

Epidemiologic studies may be grouped into four types: (1) case reports; (2) case-control studies; (3) cohort studies; and (4) clinical trials. These are placed in ascending order of their effectiveness in ruling out alternative hypotheses; that is, case reports are the least effective and clinical trials are the most effective designs. Advantages and disadvantages of the first three types of studies are discussed below.

Case Reports In human developmental toxicology, case reports fall into one of two types. The first type is what is naturally thought of as a "case" (i.e., a report of an affected fetus or infant). Usually the end point is a rare or unique aberration. In these instances, the investigator searches for previous examples in the literature and past exposures that might be linked to the incidence of this case. The analytic technique is "luck," in that it is hard to ask the right question to elicit a meaningful response regarding exposure.

The second type of case report is one in which exposure to a suspected developmental toxicant has occurred during pregnancy, either because of treatment for an existing illness or because of inadvertent exposure prior to the knowledge that a woman is pregnant. In this instance, the outcome of the pregnancy is of interest, regardless of what it is. If there are no observable effects from the exposure, this information might be useful in establishing a lower limit for exposure that does not adversely affect the conceptus. If there is an adverse outcome, an hypothesis might be developed that the exposure caused the effect.

A causal hypothesis is very difficult to establish through case reports alone, however. In the first place, case reports are dependent on the practitioner's interest and ability to perceive the importance of the situation. In addition, published reports depend on the willingness of editors to publish. Hence, publication of case reports tends to be haphazard and the information provided tends to be incomplete. Also, it should be noted that without a comparison series, it is not possible to determine what the expected rate of adverse effect should be.

As a method of detecting human developmental toxicants, reliance on case reports has gained widespread recognition due to the historical discovery of such developmental toxicants as thalidomide and alcohol. These were followed by other epidemiologic and laboratory studies in order to establish a cause and effect relationship. In general, case reports are most useful for generating hypotheses to be tested in further studies.

Case-Control Studies Case-control studies differ from case reports by the addition of a simultaneous series of unaffected individuals. Parents of both affected and unaffected offspring are interviewed, their records are searched, and external reports are examined, when available, to establish whether the infants had been exposed *in utero* to one or another suspected developmental toxicant. The ratio of exposure rate among the cases to exposure rate among the controls is the odds ratio, which, for rare conditions, closely approximates the relative risk (ratio of disease rate among the exposed to the disease rate among the unexposed). If the condition is rare, the case-control approach provides a more rapid and cost-effective method of assessing risk than does the cohort design. Log-linear methods of estimating odds ratios allow for statistical control of potential confounding factors.

Case-control studies are end-point-specific; that is, they are studies of cases demonstrating effects or lack of effects on a particular end point. If an exposure affects more than one end point, several case-control studies would be required to investigate all its effects. The importance of this last problem becomes apparent when one begins to compare end points between animal and human studies.

Cohort Studies Cohort studies of developmental effects involve observation of a group of exposed pregnant women and another group of unexposed pregnant women. The rates of adverse outcomes for both groups are compared, using relative risk analysis, sometimes controlling statistically for other differences between groups with a multivariate analytic technique.

One advantage of the cohort design is that all pregnancy outcomes may be examined, including anatomical malformations, spontaneous abortions, intrauterine growth retardation, and developmental deficits. This advantage is limited, especially for the less frequent outcomes, by the size of cohorts being followed and by the length of follow-up.

Cohort size may be restricted due to the limited number of individuals exposed in the population. Even if the entire population were at risk of exposure, the primary subgroup of interest for investigation would be the female half of the population potentially exposed during pregnancy. This often results in small sample sizes providing marginal or inadequate statistical power, and also makes it difficult to control for many potentially confounding variables.

Clinical Trials The experimental arm of epidemiology is the randomized clinical trial. However, to date it has not been used extensively in assessing developmental toxic effects, because trials would not be ethical or practical for studying environmental chemicals, including pesticides.

Confounding Factors

Factors that affect a study's usefulness for risk assessment include: (1) the power of the study; (2) potential bias in data collection; (3) the control of potential risk factors, effect modifiers, and confounders; and (4) statistical factors.

The Power of the Study The power, or ability of a study to detect a true effect, is dependent on the size of the study group, the frequency of the outcome in the general population, and the level of excess risk to be identified. In a cohort study, common outcomes, such as recognized fetal loss, require the study of hundreds of pregnancies to yield a high probability of detecting a modest increase in risk (e.g., 133 in both exposed and unexposed groups to detect a two-fold increase; alpha = 0.05, power = 80%); the probability of detecting less common outcomes, such as the total of all malformations recognized at birth, requires the study of thousands of pregnancies (e.g., more than 1,200 in both exposed and unexposed groups) to have the same statistical power (Bloom, 1981; Selevan, 1981, 1985; Sever and Hessol, 1984). In case-referent studies, study sizes are dependent upon the frequency of exposure within the source population.

A posteriori determination of power of the actual study is useful in evaluating negative findings. Negative findings in a study of low power would be given considerably less weight than either a positive study or a negative study with high power.

Potential Bias in Data Collection Sources of bias can include selection and information (Rothman, 1986). Selection bias occurs when an individual's willingness to participate varies with certain characteristics relating to the exposure status or health status of that individual. In addition, selection bias can operate in the identification of subjects for study. For example, in studies of very early loss of the conceptus, use of hospital records to identify embryonic or early fetal loss will underestimate events, because women are not always hospitalized for, or even aware of, these outcomes. More weight should be given to a study in which a more complete list of pregnancies is obtained by, for example, either interviewing the women in the study or, in a prospective study, collecting biologic data (e.g., hCG measurements) of pregnancy status from study subjects. A second example of different levels of ascertainment of events is the use of hospital records to study congenital malformations. Hospital records contain more complete data on malformations than do birth certificates.

Information bias can result from misclassification of characteristics of individuals or events identified for study. Differential misclassification (i.e., when certain subgroups are more likely to have misclassified data than are others) can either raise or lower the risk estimate. Non-differential misclassification will bias the results toward a finding of "no effect." One type of information bias, recall bias, occurs when respondents with specific exposures or outcomes recall information differently than those without the exposures or outcomes. Interview bias might result when the interviewer knows *a priori* the category of exposure (for cohort studies) or outcome (for case-referent studies) in which the respondent belongs. Use of highly structured questionnaires with or without "blinding" of the interviewer will reduce the likelihood of such bias.

When data are collected by interview or questionnaire, the appropriate respondent depends upon the type of data or study. For example, a comparison of husband-wife interviews on reproduction found the wives' responses to questions on pregnancy-related events to be considerably more complete and valid than those of the husbands (Selevan, 1980; Selevan et al., 1982).

Data from any source may be prone to errors or bias. Validation with an independent data source (e.g., vital or hospital records) or use of biomarkers of exposure or outcome, where possible, may indicate the degree of bias present and increase confidence in the results of the study.

Control of Potential Risk Factors, Effect Modifiers, and Confounders Potential risk factors include smoking, alcohol consumption, drug use, past reproductive history, genetic predisposition, pathologic conditions, and environmental and occupational exposures. Such characteristics should be examined, where appropriate, for the outcome under study, and controls for these factors should be incorporated in the study design or analysis.

The potential for characteristics of the subjects to be either effect modifiers or confounders, or both, also should be considered. An effect modifier is a factor that produces different exposure-response relationships at different levels of the effect modifier. For example, maternal age would be an effect modifier if the risk associated with a given exposure increased with the mother's age. A confounder is associated with both the exposure and outcome, and these interrelationships could distort both the magnitude and direction of the measure of association between the exposure of interest and the outcome. For example, smoking might be a confounder in a study of the association of socioeconomic status and low birth weight, since smoking has been associated with both.

Statistical Factors As in animal studies, pregnancies experienced by the same woman are not independent events. In animal studies, the litter is generally used as the unit of measure to deal with this. This approach is difficult in humans since births are not usually multiple, and the risk factors change for different pregnancies (IRLG, 1981; Kimmel et al., 1986; McMichael, 1976; Selevan, 1981, 1985). If more than one pregnancy per woman is included, as is often necessary due to small study groups, the use of nonindependent observations overestimates the true size of the population at risk and artificially increases the significance level (Stiratelli et al., 1984). Some approaches to deal with these issues have been suggested (Kissling, 1981; Selevan, 1985; Stiratelli et al., 1984). To date, a generally accepted solution to this problem has not yet been developed.

COMPARISON OF HUMAN AND ANIMAL DATA

Assumptions and Limitations in the Comparison of Human and Animal Data

Kimmel et al. (1986), Hemminki and Vineis (1985), and Nisbet and Karch (1983) state several assumptions and limitations that were similar in their evaluations of the predictability of animal data for human developmental toxicity. These are summarized below:

- Only those agents that were demonstrated to have a positive effect in man and for which there is information in both man and animals can be evaluated for concordancy of effects. However, even with this constraint, reports of an association between exposures and developmental toxicity in man are not equally convincing for the existence of a causal link. Studies may have many different objectives, from hypothesis generation to hypothesis confirmation, so that studies must be carefully scrutinized.

- The demonstration of a lack of effect in man requires an amount and quality of epidemiologic evidence that is very difficult or impossible to attain.

- Multiple classes of end points (i.e., malformations, death, growth retardation and functional impairment) may be regarded by some as different degrees or types of developmental toxicity responses. Although it was acknowledged that all end points should be considered, Hemminki and Vineis (1985) restricted their review to structural malformations because of the small size of the data base on all end points, and because most analytical epidemiologic studies are of the case-control type, in which only one effect is taken into consideration.

- Manifestations of prenatal toxicity were presumed to be variable among species; i.e., an animal model cannot be expected to mimic exactly the response in man. Also, the human population has exhibited an array of responses, which are determined by magnitude of exposure, timing of exposure, inter-individual differences in sensitivities due to genotype, interaction with other types of exposure and interaction among all of these factors. Just as man and rat are not the same, not all human subjects respond identically to exogenous influences.

All three of the reviews made qualitative comparisons of the human and animal data available. These qualitative comparisons involved not only whether an effect was reported, but also what type of effect. Several types of effects were usually considered in the animal studies, although as indicated above, Hemminki and Vineis (1985) restricted their evaluation to structural malformations and did not include drugs in the agents considered.

Kimmel et al. (1984) and Nisbet and Karch (1983) also made quantitative comparisons of a small number of agents. In these comparisons, the lowest effect levels were compared among species for the few chemicals for which such data were available. This was important because there is still not adequate dose-response information to compare sensitivities across species for most established human developmental toxicants. Hemminki and Vineis (1985) also did a semi-quantitative comparison of a few chemicals, but it does not appear from their article that the animal data were critically reviewed.

Overview of Findings Regarding Comparability

The three reviews present their findings in somewhat different ways. For example, Nisbet and Karch (1983) summarize their qualitative comparison for 11 chemicals by indicating reported sites of defects in humans and in animals (Table 7-1). They indicate that with two exceptions (anesthetic gases and warfarin), there are close parallels in the results of animal experiments to the effects reported in humans. Kimmel et al. (1984) presented a listing of those agents that show reasonable evidence of being human developmental toxicants, compared with species in which the same agents have also been effective. Also, they indicate in which species the earliest report of adverse developmental effects was reported in the literature. It appears that qualitative indicators of effect were available from animal studies for most of the agents evaluated before they were recognized in humans. There did not appear to be unique differences among species in the types of effects displayed, with perhaps the single exception of thalidomide.

TABLE 7-1. Comparison of Reported Teratogenic Effects of 11 Agents in Humans and in Experimental Animals[1]

Agent	Reported Effects or Sites in Humans	Reported Effects or Sites in Animals
Anesthetic gases	Hemangiomas, hernias, skin, heart	Skeletal defects only: rat, mouse halothane and N_2O)
Smelter emissions (lead and/or arsenic)	Multiple malformations	Multiple malformations rat, mouse, hamster (lead and arsenic)
Polychlorinated biphenyls	Skin discoloration; enlarged fontanelles	Skin discoloration and lesions: rhesus monkey; enlarged fontanelles and syndactyly: pig, dog, negative: rat, rabbit
Alcohol	Facial, CNS	Facial, dermal, neural, extremities: rat, mouse
Vinyl chloride	Neural tube	Various, including encephalocele: rat
Warfarin	Nose, bones (case reports only)	Negative: mouse, rabbit
Diphenylhydantoin	Cleft lip, cleft, palate, other craniofacial, mental deficiency	Cleft lip, cleft palate, syndactyly, other skeletal defects: mouse; minor kidney anomalies: rhesus monkey
Aminopterin	Multiple malformations	Multiple malformations: sheep, rat
Busulfan	Eye, cleft palate (1 report)	Skeletal, genital defects: rat
Methotrexate	Skull, ribs, toes (2 reports)	Various: rat, cat, rabbit, mouse
Methylmercury	CNS	CNS, skeletal: rat, mouse, hamster, cat

Hemminki and Vineis (1985) present both a qualitative and quantitative comparison for six chemicals (Table 7-2). Although some substances induced different types of lesions in humans and experimental animals, and the exposure and dose-response data in humans were often crude or inadequate, developmental effects were seen in one or more animal species tested that could be used to predict a response in humans.

Thus, all the reviews suggest that on a qualitative basis, at least one species predicted adverse developmental effects similar to those seen in humans for most chemicals. If the comparison is made based on whether or not any type of developmental effect was seen in the animal studies, then a more general conclusion can be drawn that almost all species tested showed some evidence of a developmental effect. This is encouraging for the use of experimental animal studies in predicting risk for human developmental toxicity, since it is impossible to know *a priori* which will be the most appropriate species for predicting specific types of effects in humans.

Nisbet and Karch (1983) present a summary for comparison of the lowest effective doses of eight teratogens in humans and animals. Kimmel et al. (1984) present a similar summary for five chemicals (Table 7-3). A few chemicals appear on both lists, but the doses and species used for comparison were not always the same. However, similar conclusions may be drawn concerning these data. It appears that, for almost all chemicals, the ratios of the lowest effective doses in animals and humans were within an order of magnitude, even though differences in experimental conditions employed often preclude accurate comparison. Few additional human teratogens have been identified since the above summaries were completed (e.g., valporic acid, retinoic acid), and the Teratology Society (1987) presented a summary indicating that humans are as much as 100 times more sensitive to retinoic acid than the most sensitive animal species (i.e., mouse).

Overall, the findings of these reviews indicate that there is a relatively small data base on which to establish both qualitative and quantitative comparisons; however, it appears that animal studies are generally predictable for most agents in a qualitative way. Further efforts should be focused on increasing the data base with careful epidemiologic studies that include good exposure information and dose-response data, if possible. Improving the precision of the animal findings, that is, quantifying the exposure and the response, as well as adding information on pharmacokinetics and mechanisms of action, should help to improve interpretation of both human and animal data and extrapolation of risk from animal studies to humans.

TABLE 7-2. Animal Tests on Chemicals with High and Limited Evidence of Teratogenicity in Man After a Measured Exposure

Substance	Daily Dose (mg/kg)	Risk	Reference	Species	Daily Dose (mg/kg)	Risk
Carbon Monoxide	Unknown	CNS defects	[4.31.32]	Mouse, rat, rabbit	30-250 ppm	Resorptions
Ethanol	2,000	33% (FAS)	[37.40.41]	Mouse	3,000-5,000	30% (FAS type)
Halothane[a]	60 ppm = 0.6 ml/kg.	1.5 times	[9-23.156]	Mouse	1.10 1/kg	Threefold (skeletal)
Methylmercury	1.0[b]	25% microcephaly	[65]	Mouse, rat	5-10	50% Malformations
				Cat	0.25	32% Malformations
Nitrous Oxide[c]	3,000 ppm-0.31 l/kg	5.5 vs. 3.6% risk	[13.156]	Rat	300 l/kg.	Malformations sixfold
Polychlorinated Biphenyls	0.5[d]	100% (symptoms) 30% (small for date)	[780.71.73]	Rat, monkey	0.2 l/kg. 0.10	Fetal Deaths: 13% skin symptoms, 50% low birth weight, 40% lethality

[a] It is assumed that halothane is the responsible agent in anesthetics; in fact, the evidence is inadequate.
[b] For methylmercury, 1 mg/kg is the total ingested dose; the daily doses were in the order of 80 μg/kg.
[c] It is assumed that nitrous oxide is the responsible agent in anesthetics; in fact, the evidence is low.
[d] Estimated intake of Kanechlor.

TABLE 7-3. Comparisons of Effect Levels for Teratogens in Man and Test Species

Agent	Species	Dose	Reported Effects
Aminopterin	Man	0.1 mg/kg/da	Death/malformations
	Rat	0.1 mg/kg	Death/malformations
Diethylstilbestrol	Man	0.8-1.0 mg/kg	Genital tract Abnormalities/death
	Mouse	1 mg/kg	Genital tract Abnormalities/death
Ionizing radiation	Man	20 rads/da	Malformations
	Rat/Mouse	10-20 rads/da	Malformations
Cigarette smoking	Man	20 cigarettes/da	Growth retardation
	Rat	>20 cigarettes/da	Growth retardation
Thalidomide	Man	0.8-1.7 mg/kg	Malformations
	Monkey	5.0-45 mg/kg	Malformations
	Rabbit	150 mg/kg	Malformations

RISK ASSESSMENT FOR DEVELOPMENTAL TOXICITY

The National Research Council (NRC, 1983) has defined risk assessment as being comprised of some or all of the following components: hazard identification, dose-response assessment, exposure assessment, and risk characterization. In general, the process of assessing the risk of human developmental toxicity may be adapted to this format. However, many aspects of hazard identification and dose-response assessment are conducted together in practice.

Hazard identification and dose-response assessment involve the evaluation of all available experimental animal and human data to determine the conditions (dosage, timing, species, strain, etc.) under which an agent is likely to cause any type of developmental toxicity. In considering developmental toxicity, not only structural abnormalities should be evaluated, but also fetal wastage, growth alteration, and functional abnormalities that might result from developmental exposure to environmental agents.

The exposure assessment identifies human populations exposed to the agent, describes their composition and size, and presents the types, magnitudes, frequencies, and durations of exposure to the agent. The exposure assessment provides an estimate of human exposure levels from all potential sources.

In risk characterization for humans, the exposure assessment and the hazard identification and dose-response assessment are combined to estimate some measure of the risk of developmental toxicity. The no-observed-adverse-effect-level (NOAEL) and lowest-observed-adverse-effect-level (LOAEL) in human and laboratory animal studies and the estimated human exposure levels are compared (margin of safety) to determine the acceptability of a human exposure level. As part of risk characterization, a summary of the strengths and weaknesses in each component of the risk assessment are presented together with major assumptions, scientific judgments, and, to the extent possible, estimates of the uncertainties.

Risk assessment is just one component of the regulatory process and defines the adverse health consequences of exposure to a toxic agent. The other component, risk management, combines risk assessment with the directives of the pertinent enabling regulatory legislation, together with socioeconomic, technical, political, and other considerations, to reach a decision as to whether or to what extent future exposure to the pesticide is controlled. For example, the pesticide law (FIFRA) requires EPA to weigh the risks and benefits of a pesticide use. Therefore, the same risks which are not considered unreasonable for a highly beneficial product or use may be found unreasonable for a less important use.

Weight-of-Evidence Determination

Information available from studies discussed previously, whether indicative of potential concern or not, must be evaluated and factored into the risk assessment. The primary considerations are the human data (which are seldom available) and the experimental animal data. The qualitative assessment for developmental toxicity should consider quality of the data, resolving power of the studies, number and types of end points examined, relevance of route and timing of exposure, appropriateness of the dose selection, replication of effects, number of species examined, and availability of human case reports, case series, and/or epidemiologic study data. In addition, pharmacokinetic data and structure-activity considerations, as well as other factors that may affect the strength of the evidence, should be taken into account. Therefore, all data pertinent to developmental toxicity should be examined in the evaluation of a chemical's potential to cause developmental toxicity in humans and sound scientific judgment should be exercised in interpreting the data in terms of the risk for adverse human developmental health effects.

The proposed 1989 amendments to the EPA Guidelines for the Health Assessment of Suspect Developmental Toxicants (EPA, 1989a) provide a weight-of-evidence scheme for developmental toxicity. This scheme defines three levels of confidence for data regarding identification of potential developmental hazards: definitive evidence, adequate evidence, and inadequate evidence. Within the definitive evidence and adequate evidence categories, there are subcategories for evidence indicating adverse effects and for evidence indicating no apparent effects under specified conditions of exposure, etc. In both categories, the evidence required to classify an agent as demonstrating no adverse effects is greater than that required to demonstrate an adverse effect and must include evaluations of all potential manifestations of developmental toxicity. Greater evidence is required because it is much more difficult both biologically and statistically to support a finding of no apparent adverse effect than one of an adverse effect. Most agents meeting current testing requirements would be expected to fall within the adequate evidence category, while many for which little or no information is available would be classified in the inadequate category. Few agents would be expected to fall into the definitive evidence category because the human data necessary for this category would be difficult to obtain.

Placing an agent in a particular weight-of-evidence category such as "adequate evidence for human developmental toxicity" does not mean that it will display developmental toxicity at every dose (because of the assumption of a threshold) or in every situation (e.g., hazard may vary significantly depending on route and timing of exposure). Thus, the weight-of-evidence determination should always be presented in conjunction with information on dose-response (NOAEL and/or LOAEL), and, if possible, with the human exposure estimate (margin of safety).

Exposure Assessment

The results of the dose-response assessment are combined with an estimate of human exposure to obtain a quantitative estimate of risk. In general, the exposure assessment describes the magnitude, duration, schedule, and route of exposure. This information is developed from monitoring data and from estimates based on modeling of environmental exposures. Unique considerations related to stage of development (i.e., critical periods), and the possibility that a single exposure may be sufficient to produce adverse developmental effects (i.e., chronic exposure) are not prerequisites for developmental toxicity to be manifested. Also, it should be recognized that exposure of almost any segment of the human population (i.e., fertile men and women, the conceptus, and the child up to the age of sexual maturation) can lead to risk to the developing organism.

Data on exposure of humans can be qualitative or quantitative. The qualitative data could be surrogate data, such as employment or residence histories; quantitative or dose data are frequently not available. Pesticide exposures are often estimated from surrogate data bases. The exposure of a person mixing agricultural pesticides can be estimated by using actual exposure measurements. Methods such as residue analysis from patches on the skin, air sampling in the breathing zone, or urinary monitoring for metabolites are commonly used. Those data may then be used to predict exposure to another person handling a different product that has similar physical and chemical properties and uses similar equipment.

Exposures at different stages of the reproductive process can result in different outcomes (Wilson, 1973). In laboratory studies, these time periods can be carefully controlled. In human studies, especially retrospective ones, linking of specific time periods and specific exposures, even on a qualitative level, may be difficult due to errors of recall or record keeping (if records are available). The increased probability of misclassification of exposure status may affect the ability of a study to recognize a true effect (Hogue, 1984; Kimmel et al., 1986; Lemasters and Selevan, 1984; Selevan, 1981a; Sever and Hessol, 1984).

For assessing the developmental toxicity of pesticides, EPA regulators are generally concerned about the highest daily exposures. Their concern is that developmental effects might result from a single exposure to a pesticide at a critical period of development. They regulate on the basis of ranges of exposure and use the upper-bound (95 percent) confidence limit for exposure. This approach ensures that the person handling the pesticide is protected against the occasional, but reasonably likely, higher exposure.

Dietary exposures to pesticides are based upon either maximum permissible residues, such as the EPA tolerance level, or, when available, actual pesticide residues on food as eaten. The upper confidence limit for residues is considered in estimating margins of safety.

Risk Characterization

In risk characterization, the dose-response and exposure data may be combined to calculate the margin of exposure. The margin of exposure approach derives a ratio of the NOAEL from the most sensitive species to the estimated human exposure level (EPA, 1985). The adequacy of the margin of safety is then considered, based on the weight of evidence, including the nature and quality of the hazard and exposure data, the number of species affected, dose-response relationships, and other factors such as benefits of the pesticide.

Uncertainty factors and margins of safety are conceptually similar but are computed differently and are used in different regulatory situations. The choice of approach is dependent upon many factors, including the statute involved, the situation being addressed, the data base used, and the needs of the decision-maker. The acceptability of the final uncertainty factor used or the acceptability of the margin of safety are risk management decisions, but the scientific issues that must be taken into account in establishing them have been addressed here.

Of pesticides considered to be developmental toxicants, such as dinoseb (EPA, 1987), recent regulatory actions have indicated that a margin of safety (or margin of exposure) of 100 will normally be acceptable at contemporary use levels. Although 100 is listed as a "rule of thumb," lower margins may result from careful scrutiny of the benefits of the pesticide.

SUMMARY AND CONCLUSIONS

The presence of chemicals and drugs has been shown to produce developmental effects in humans, but cause and effect association between exposure and toxicity are difficult to make. The evidence presently available supports the tentative conclusion that exposure to levels of environmental chemicals currently considered acceptable is not observed to be a threat to human development. However, absence of an observed cause-and-effect relationship can be a matter of the detecting methods employed. Furthermore, it is important to recognize that while the developing embryo might not be injured by the majority of agents except at exposure levels sufficiently high to produce acute maternal toxicity, human exposures can occur at such levels. An undetermined minority of chemicals are capable of interfering with development at a small fraction of the maternally toxic exposure levels. Even though Warburton (1987) has pointed out that some degree of reproductive loss is normal, increased awareness of chemical selectivity and severity of maternal toxicity is warranted to detect and evaluate more fully the effects in exposed human populations.

Reports available in the published literature indicate that there are approximately 1,400 active ingredients in roughly 60,000 pesticide formulations used worldwide (Schardein, 1985). These are divided into four classes: fungicides, herbicides, insecticides, and miscellaneous pesticides (fumigants, rodenticides, parasiticides, etc.). Even without consideration of study quality, dosing period, or level of exposure, the majority of the fungicides as reported by Schardein had been found to produce developmental defects in experimental animals.

The same general conclusion is possible from published reports of herbicides or insecticides tested in pregnant laboratory animals. During consideration of such reports, it is essential that attention be given to the level of dose administered in each test. Some agents positive in one species were negative in others (usually tested at different treatment levels), and the majority of those lacking apparent developmental toxicity were examined in only one species. The major variable is the level of exposure. Studies that predated the clearly written guidelines for developmental toxicity risk assessment evaluation in laboratory animals often tested agents only at lower exposure levels (i.e., those that did not approach the maternally toxic level) and also lack information regarding maternal status. It is common in these circumstances to find that outcome is more often related to exposure severity than it is to agent nature, and one would assume that most pesticides, if not all, would disrupt development in humans at very high exposure levels. Not unexpectedly, particularly for such a diverse group of chemicals, there was no common pattern of malformations within or among categories of pesticides that were developmental toxicants.

Human developmental toxicity due to pesticide exposure has not been assessed quantitatively in terms of exposure levels experienced by the women. Furthermore, location of a cohort of individuals exposed to a single pesticide is exceptionally difficult; since human malformations occur in as many as 5 percent of live births (Fraser, 1977), demonstration of an increase in the total malformation rate or in the incidence of a single type of malformation after a documented exposure may require a very large population of exposed individuals or ascertainment of pregnancy outcomes in women exposed very close to their own toxic level (Johnson, 1989). The majority of, but not all, human exposures to pesticides are at low doses; whereas, developmental toxicity testing protocols almost always use much higher doses. The relationship between toxicity at high and low doses, and the extrapolation of animal data to humans are two of the serious problems that have a dramatic impact on our ability to ascertain whether humans are at risk for maldevelopment from pesticides used at low exposure levels. It would be useful to examine in detail the outcome of pregnancies exposed to high,

maternally toxic levels of pesticides rather than lumping all degrees of exposure into one group for comparison with "unexposed" mothers.

FUTURE DIRECTIONS

Defense Systems

The effects of foreign organic chemicals (e.g., pesticides) on living organisms are strongly dependent upon a variety of endogenous factors possessing the potential to prevent or ameliorate toxic responses. These endogenous factors represent defense systems, the integrity of which is often a crucial determinant of the magnitude, intensity, nature, and duration of the toxic response. Such defense systems have been discussed in detail by Reed (1985) as well as by numerous others and include enzymatic detoxification, renal and biliary active transport systems, glutathione and other cellular thiols, antioxidants such as ascorbate, tocopherols, beta-carotene, uric acid, etc., and enzyme systems that protect against the effects of xenobiotic-dependent generation of reactive oxygen species (glutathione peroxidase, superoxide dismutase, catalase, etc.). Although these defense systems have been studied in adult experimental animals, the data base regarding effectiveness levels during the earlier, more critical stages of embryonic development is exceedingly sparse and in need of further expansion. Support for this premise is provided by accumulating evidence regarding the role of reactive intermediary metabolites in the genesis of developmental toxicity (Juchau et al., 1987). An understanding of the qualitative and quantitative capacity of these systems, assessed as a function of early development, is essential for definitive evaluation of the risk of chemicals convertible to such intermediates --and this includes most chemicals.

Employee Surveillance System

Shortly after the "thalidomide disaster," Wilson (1963) pointed out: "Difficult as it is, this Public Health procedure of constant surveillance of the incidence of disease must be, in the last analysis, the final protection against a similar great disaster." Although a national surveillance system is in place in the United States covering about one-third of all hospital births (Edmonds, et al., 1981), this system has been criticized for a variety of reasons, including the lack of information about exposure to toxic substances (Levin, 1983).

A thought commonly expressed by those without first-hand experience is that workers in the chemical industry would be the population most likely to be chronically exposed to the highest levels of chemicals and would more likely experience toxicity from specific chemicals than the average individual. Thus, surveillance of chemical industry workers would be expected to more efficiently identify exposures to substances that are injurious to development than surveillance of larger, more general populations. Therefore, it would be useful to have a system of surveillance of chemical industry workers exposed to potentially toxic substances, with "non-exposed" workers serving as controls. er, those with first-hand experience realize this approach would probably not yield the positive data sought.

The highest exposure levels tend to occur in "mom and pop" operations and due to accidents. There have been a few accidents in "the chemical industry" but most occur away from the plant site (e.g., during transport, and during or immediately after improper storage, application, or use). Chronic exposure may be more prevalent among some employees in the chemical industry; however, this would only be very low levels as good hygiene is commonly practiced in today's world. Large and mid-sized companies, as well as most contractors, have monitoring and medical surveillance in place which includes annual physical examinations for employees. Except for possibly pharmaceuticals, more is known about the health effects of pesticides than for virtually anything else. Also, the number of fertile women among workers in manufacturing operations is very small, especially on a global basis. Furthermore, under current regulations if anyone is aware of a potentially harmful exposure condition in the workplace under threat of criminal prosecution, it must be reported to the appropriate government agency, and corrected even if it might mean termination of the process.

For pesticides, there is general concern regarding exposure levels that might occur among applicators, loaders, mixers and laborers, if precautions on the labels are not adhered to. Also, there is a concern regarding the general population, including homeowners, that may not take adequate precaution or practice adequate hygiene during and after use of pesticides. It may be in these circumstances that the conceptus is at highest risk from pesticide exposure.

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